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Phylogenetic Relationships of Drug-Resistance Factors and Other Transmissible Bacterial Plasmids

ELINOR MEYNELL, G. G. MEYNELL, AND NAOMI DATTA

Medical Research Council Microbial Genetics Research Unit, Hammersmith Hospital, London, W. 12; Guinness-Lister Research Unit, Lister Institute of Preventive Medicine, London, S.W.1; and Department of Bacteriology, Royal Postgraduate Medical School, London, W.12, England

INTRODUCTION

The drug-resistance (R) factors found in *Enterobacteriaceae* possess two outstanding functions. They render their hosts resistant to antibacterial agents such as antibiotics, and, at the same time, enable them to transmit resistance to other bacteria. The recipient of an R factor thus becomes drug-resistant and a genetic donor in its turn. It may also receive other genetic material from the donor cell, such as segments of chromosome or other extrachromosomal elements which the donor may carry at the time. Chemically, R factors consist of deoxyribonucleic acid (DNA), like the bacterial chromosome, although they behave independently in biophysical (186) and genetic experiments. An R factor is thus an independent linkage group, composed of genes determining drug resistance associated with others conferring the ability to conjugate and to transfer the factor to a new host. The latter have been collectively referred to as the "resistance transfer factor" or RTF (219), or "transfer factor" (7), but, as those found in R factors are unlikely to differ essentially from those possessed by other types of transmissible plasmidlike colicin factors' a more general term is to be preferred. Of the terms available, "sex factor" seems at present the most suitable and is used here, although, after it was first applied to the F factor, its meaning has been both extended to any transmissible plasmid as a whole (97, 116) and restricted to those plasmids capable of promoting chromosomal transfer (33).

The phenomenon of transmissible drug resistance was discovered in Japan (219), following the isolation from a case of dysentery of a strain of *Shigella flexneri* resistant to the four drugs, chloramphenicol, tetracycline, streptomycin, and sulfonamide (121). Multiply resistant *Shigella* strains were subsequently found with increasing frequency in clinical practice in Japan, and became of particular interest when it was observed that, after administration of a single antibiotic, both fully sensitive and multiply resistant strains belonging to the same serological type could be isolated in specimens from either a single patient or different patients in the same outbreak. Akiba (3, 4) and Ochiai (168) suggested that the resist-

ance might be transferred to *Shigella* from strains of *Escherichia coli* already resistant, and showed that multiple resistance of this kind was indeed transmitted from one bacterium to another by cell contact. Watanabe and Fukasawa (222) confirmed that cell contact was required for the factor to bring about its own transfer, although the resistance genes could also be transduced by phage (223). Many reports followed, from 1957 onwards, of strains of *S. flexneri* or *E. coli* resistant to one or more antibiotics whose resistance was similarly transmissible by growth in mixed culture (155). Transmission did not depend on the presence of F, the classical sex factor of *E. coli* K-12 (157), so that the bacteria had evidently acquired an independent mechanism for conjugation.

The first R factors to be reported outside Japan were found by Datta (48, 49) in strains of *Salmonella typhimurium* causing an outbreak of gastroenteritis in London. Since then, it has become apparent that transmissible drug resistance is prevalent in strains of *Salmonella*, *Shigella*, and *E. coli* throughout the world (7, 124, 130, 143, 144, 200, 201). Resistance can be transferred widely among the *Enterobacteriaceae* (91), *Vibrio cholerae* (16, 126), *Pasteurella pestis* (80), and *Serratia marcescens* (186), although the recipient ability of a strain may be limited by restriction on the entry of foreign DNA (124; M. Okada et al., J. Gen. Microbiol., *in press*).

The R factors which were first discovered conferred resistance to one or more of the drugs, streptomycin, tetracycline, chloramphenicol, and sulfonamide (158, 219); resistance has subsequently appeared to kanamycin and neomycin (130) and to the penicillins (8), while strains are not uncommonly found which transfer resistance to all these drugs (125). Since 1960, R factors have accumulated an increasing number of drug-resistance determinants (7, 70) and have also become increasingly prevalent in enteropathogenic species (salmonellae, shigellae, and enteropathogenic strains of *E. coli*). Their incidence in nonpathogenic bacteria has not been investigated to the same extent.

It has been the custom, as new transmissible plasmids are discovered, to group them by the more obvious properties they confer on the host bacterium. In this way, R factors are generally considered as a class determining resistance to antibiotics, as distinguished from plasmids associated with other characters, like colicinogeny. It is questionable, however, whether this is a useful distinction, for we cannot judge the significance of resistance and other genes until we have discovered their ultimate source and how they were acquired. The sex factor, or that part of the plasmid responsible for its transmissibility, may be of far greater significance in any system of classification (28). In attempting to determine the fundamental nature of complex plasmids, we should perhaps look first for those factors which confer no other character on the bacterium beyond the ability to conjugate, for it may be an uncomplicated sex factor of this kind which is the rudiment of the future complex plasmid (6, 150). The obvious example is the F factor itself; other instances are the factors described by Lederberg, Cavalli, and Lederberg (132), Bernstein (18), Furness and Rowley (78), Ørskov and Ørskov (171), and Anderson and Lewis (10). Any attempt, however, to relate the habitual incidence of such sex factors to the frequency with which R factors are found is likely to be vitiated by the fact that once an R factor is constituted, the sex factor will be subject to the same selective pressures from the use of antibiotics as the resistance determinants themselves. This prediction has been borne out in *Salmonella panama* (82, 83) and in *S. typhimurium* (6, 7) by a prodigious increase in the prevalence of certain phage types, for the phage resistances which define these particular types depend on sex factors which serve as the vectors of drug-resistance genes.

Far more is known at present of the sex factors of R factors than of the origin of their resistance determinants. By the time that transmissible drug resistance was discovered, a great deal was already known about conjugation in enterobacteria from studies of the F factor of *E. coli* K-12 (96, 116, 132), and of colicin factor I (ColI), another genetic element known to bring about conjugation and the transfer of genetic material (74, 175). This factor was evidently quite distinct from F, judging from their differing behavior (Table 1). In particular, ColI brought about its own transfer at much lower frequency than did F (207) and gave only a very small number of genetic recombinants for chromosomal genes (175); neither could the surface properties associated with F be detected in F^- cells carrying ColI. But the most striking feature of ColI transfer was the HFT (high-frequency transfer) system, where bacteria which had newly received the factor passed it on at much higher frequency than those which had carried it for many generations (207). These differences between F and I (as the sex factor of ColI will be called) arise both from differences in the structural genes determining conjugation and in the degree to which these genes are repressed in the two factors.

The sex factors of naturally occurring R factors appear to be related to F or to I by a variety of criteria, so that F and I may well be the archetypal sex factors from which the majority of trans-

TABLE 1. *Properties of F and I*

Factor	Wild-type factor is	Rate of transfer per cell		Type of sex pilus	Pilus detectable in established cultures[b]
		Plasmid[a]	Chromosomal genes[a]		
F	Derepressed	0.2–1	10^{-4} to 10^{-5}	F	+
I	Repressed	10^{-3} to 10^{-5} (LFT) 0.1 to 1.0 (HFT)	10^{-8} to 10^{-9}	I	−

[a] In *Escherichia coli* K-12 (37, 175).
[b] By electron microscopy, agglutination by antiserum, macroscopic lysis by phage.

missible plasmids have originated. In outline, the F-like group contains F and its derivatives, *fi*+ R factors, some *col* factors, and various other plasmids such as F0-*lac*. The I-like group comprises other colicin factors, notably ColI, and *fi*− R factors.

F AS A MODEL SEX FACTOR

It is now 22 years since genetic material was first shown to be transferred from one bacterium to another by conjugation following cell contact (134, 214). Not long after, it became apparent that, although the genes transferred were located in the bacterial chromosome, the actual process of transfer was determined by an additional genetic element. This element, named F for "fertility" (31), provided the cell with a mechanism for conjugation which allowed it to act as a genetic donor (96), so that, in a mating pair, one cell acted as donor and the other as recipient (95). The presence of F is recognized by two principal effects: first, the cell becomes a genetic donor; second, it produces a special filament (Fig. 1) which, as it is not concerned in motility, has been classified as a new member of that heterogeneous class of appendages known as "fimbriae" (59, 60) or "pili" (21). The pili previously described are not directly concerned in gene transfer, although they may play a nonspecific role by stabilizing pairs of conjugating cells (152, 160). These pili will be termed "common pili" (152) to distinguish them from the "sex pili" determined by F and other sex factors, which may provide the actual route of gene transfer (22).

The F pilus is detectable on F+ or Hfr cells by the appearance of a new antigen (106, 170) and because it is the receptor for certain phages which exclusively lyse cultures of bacteria carrying F. F-specific phages are of two kinds (Fig. 5, 6): isometric phages with ribonucleic acid (RNA) (237) which attach to the side of the pilus (23, 40) and filamentous phages containing single-stranded DNA which attach to the tip (29, 102). Other changes which may also be due to the F pilus are that F+ bacteria precipitate more readily than F− as the *p*H of the medium is lowered (139), have an altered surface charge, and are sometimes autoagglutinable (140). They also travel more slowly than F− cells through semisolid medium (199). The F pilus is clearly essential for conjugation, for the bacteria do not act as genetic donors if F pili are not produced (99) or are removed (22, 29), or if their tips are blocked with phage (105). The precise function of the pilus in conjugation is not yet established; it may serve as the conjugation tube through which the genes actually pass to the recipient (22) or it may simply be a means of making the initial cell contacts, so bringing the bacteria into apposition.

In a cross between F+ and F− bacteria, the number of genetic recombinants for any given chromosomal region corresponds to about 10^{-4} to 10^{-5} of the F+ population (2), although conjugation occurs very much more frequently. The frequency of conjugation may be estimated from the rate of transfer of F, measured by the appearance in the recipient of donor ability and of sensitivity to F-specific phage. It may also be measured from the transfer either of a bacterial marker forming part of a composite F′ factor (101, 109) or of the leading chromosomal marker of an Hfr strain (233). All these methods suggest that the efficiency of conjugation approaches 100% (61, 84, 101, 112, 116), which agrees with the high proportion of bacteria that show F pili in the electron microscope (22) and that are susceptible to F-specific phage (23, 51, 149). The fact that F+ and Hfr cultures are agglutinated by F-specific antiserum (170) also demonstrates that a large proportion of the bacteria must produce the specific antigen. Thus, it appears that F is expressed in virtually every F+ or Hfr bacterium; in other words, the expression of wild-type F is not repressed.

I, THE SEX FACTOR OF COLI

The marked difference between F and I has two causes (Table 1). First, the conjugating ability of ColI is subject to repression. Only 0.01 to 1%

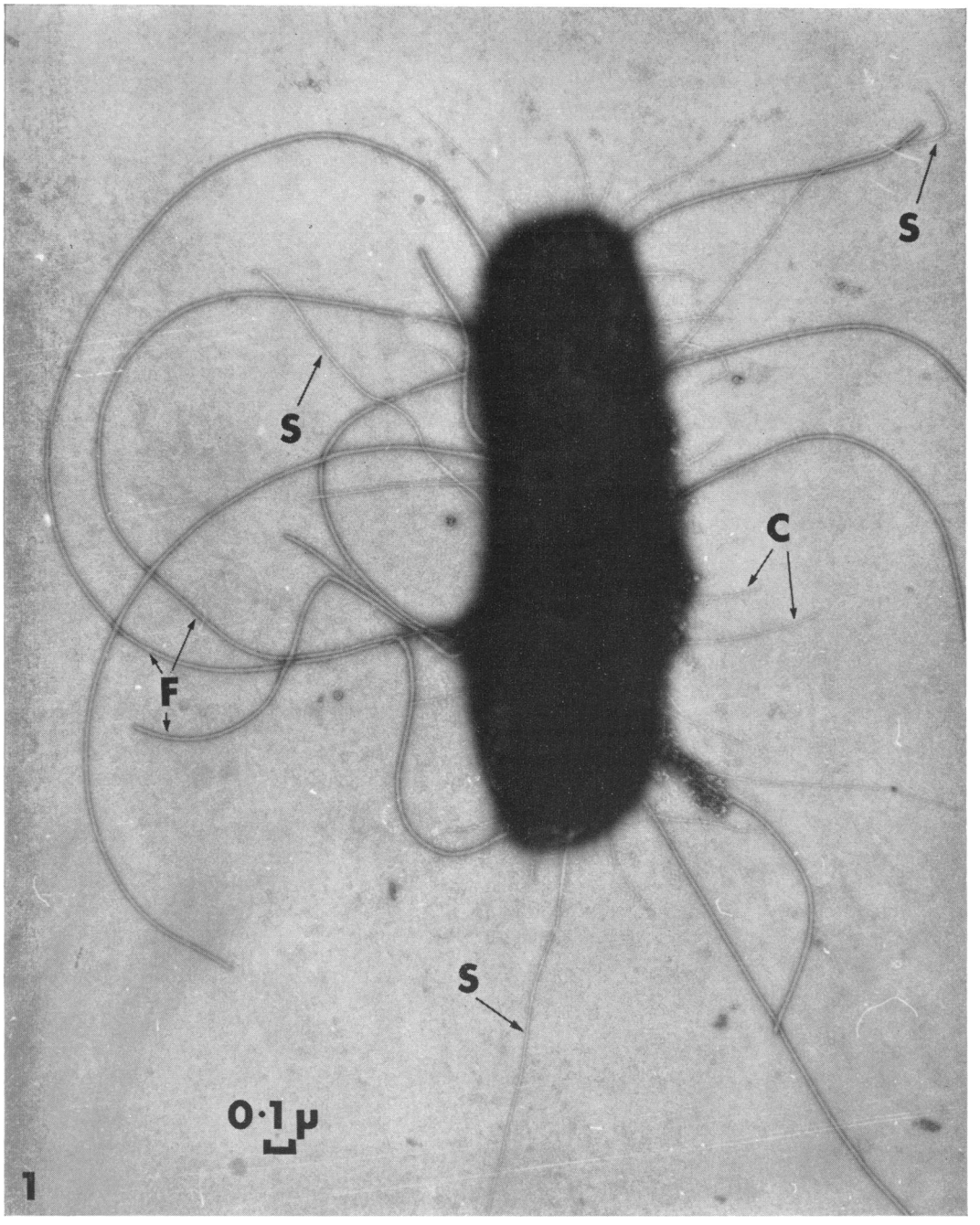


FIG. 1. *Escherichia coli, showing F-like sex pili (S), common pili (C), and flagella (F).*

of bacteria in an established ColI$^+$ culture can transfer the plasmid at a given time, so comprising a low-frequency transfer (LFT) system. However, after ColI enters a new host, it is transferred for the next three generations with up to 100% efficiency (174, 207) and then gives an HFT system. During subsequent generations, donor ability dilutes out among the multiplying bacteria until the characteristic LFT rate of transfer is again reached (174). The HFT state is analogous

to the short-lived burst of β-galactosidase synthesis that occurs in *E. coli* when the structural gene (z^+) for the enzyme is transferred by conjugation with the regulator gene (i^+) to a z^-i^- host (179); and the explanation is presumably the same in each case, namely, that the expression of sex factor activity, or z^+ gene activity, is more rapid than its repression, thus permitting full expression in the short interval before repression is established (33). The behavior of ColI transferred to ColI$^+$ recipients supports this view, for no HFT system then results, just as no β-galactosidase is formed when the z^+ gene is transferred to an i^+ recipient. In each case, the donated gene is immediately exposed to repressor formed by the factor resident in the recipient; repression is thus effective when the genes concerned are in the *trans* position, implying that it is effected by a cytoplasmic agent (113). Derepressed mutants of the I factor have recently been isolated in which conjugation function is continually expressed (S. Edwards and G. G. Meynell, *unpublished data*). The source of these factors was not ColI itself, but ColE1a, known to be associated with an I-like sex factor (153).

The second major difference between ColI and F is attributable to the sex pilus. The F pilus was originally distinguished from other bacterial appendages by its ability to adsorb isometric F phages (23, 40). However, no I phages were available when ColI$^+$ cultures were first examined by electron microscopy, and the experiments had therefore to be performed with bacterial strains that formed neither flagella nor common pili in order to reveal newly formed appendages (Fig. 4). Such strains failed to show HFT behavior when prepared in the standard way (207), and, when the spread of ColI in the cultures was examined, it was found to occur far more slowly than when common pili were formed, apparently because common pili stabilize pairs of mating cells nonspecifically (160). Once allowance was made for the slowness of spread, satisfactory HFT cultures could be produced and were found to contain a characteristic pilus that was absent from LFT or Col$^-$ cultures. This "I pilus" differs both morphologically and antigenically from the F pilus and does not adsorb F-specific phages. The I pilus does, however, adsorb I phages, which, in turn, do not adsorb to F pili. I phages have so far been isolated on only two occasions, each time from sewage (G. G. Meynell, Proc. European Phage Meeting, 1967, p. 14). Both isolates are filamentous, about 50% longer than the three filamentous F phages M13 (104), fd (103), and Ec9 (55), and contain single-stranded DNA, judging from staining with acridine orange. No isometric I phages have so far been isolated. The host ranges of F and I phages are complementary, as shown in Table 2, which also summarizes the differences between F and I sex pili. The terminal knobs mentioned in Table 2 are found only at the distal end of sex pili (Fig. 3), never on common pili (127, 129, 152); although their nature is not clear, they cannot be basal structures or "roots" (217). They are antigenically distinct from the pilus (A. M. Lawn, *unpublished data*) and may, perhaps, be cell wall extruded as the pilus emerges or fragments of cell wall picked up from the medium.

TABLE 2. *Characters of F pili, I pili, and common pili*

Pili	Maximum length	Axial canal	Terminal knobs	Clusters of pili	Phage adsorbed	
					F	I
	μ					
F-like	20.0	Often seen	+	+	+	−
I-like	2.0	Rarely seen	+	+	−	+
Common (type I)	1.5	Prominent	−	−	−	−

R FACTORS

fi$^+$ *Factors*

When transmissible drug resistance was first observed, the analogy with F was at once apparent, for here were genetic elements which brought about their own transfer by conjugation (222), and, occasionally, transfer of segments of bacterial chromosome (210). Thus, R and F both acted as sex factors but there were such marked differences in their behavior that this seemed to be the full extent of their resemblance. Conjugation with R evidently occurred far less often than with F, since R was always transferred at lower frequency; genetic recombinants for chromosomal genes were rarely observed; and R$^+$ cultures were neither agglutinated by F-specific antiserum nor lysed by F-specific phage. Moreover, the majority of R factors actually inhibited F, since introduction of an R factor into an F$^+$ strain often drastically reduced its ability to conjugate (224) and, at the same time, abolished agglutination by F-specific serum (100) and lysis by F-specific phage (225). This ability to inhibit F, named *fi*$^+$ (for "fertility inhibition") by Watanabe (227) and i^+ by Hirota, was attributed by Egawa and Hirota (62) to a cytoplasmic repressor produced by the R factor which acted on F.

After reviewing these phenomena, Meynell and Datta (148) pointed out that they might reflect, not a difference, but a close relationship between

F and the *fi*$^+$ class of R factors. The various observations were, in fact, precisely what would be expected if the *fi*$^+$ sex factor was under the control of a repressor determined by a regulator gene in the R factor *which also acted on F*. The ability of an *fi*$^+$ R factor to repress F would thus simply reflect its ability to repress its own sex factor. The view that such a repressor was produced by R factors was supported by the occurrence of HFT cultures with R$^+$ strains (220). Donor ability was also increased by exposing an R$^+$ strain to ultraviolet (UV) radiation (222), presumably owing to interference with the repressor, as in UV induction of vegetative phage growth in lysogenic bacteria.

The relationship between F and the *fi*$^+$ sex factors was examined by seeing whether R$^+$F$^-$ cells were attacked by F-specific phage, for, if the two types of sex factor were related, they should determine similar sex pili, and cells carrying *fi*$^+$ factors should therefore be susceptible to F phage. The specificity of these phages lies in adsorption, not in any later stage of phage growth, since protoplasts of F$^-$ strains of *E. coli* K-12, as well as of *Salmonella*, *Shigella*, and *Proteus*, can be infected with RNA from phage f2 (64). Thus, if the F pilus were produced by even a few cells of an R$^+$F$^-$ culture, some multiplication of F phage should be observed. Furthermore, if production of the pilus were correlated with expression of conjugating function, the proportion of phage-sensitive R$^+$ bacteria should increase in HFT cultures in parallel with the proportion of bacteria which can conjugate and transfer drug resistance. Even if the pili determined by F and by the sex factor of R were identical, the fact that they are rarely expressed in R$^+$ cultures would be quite sufficient to account for the absence of visible lysis with F-specific phage. On testing the *fi*$^+$ class of R factors, they were indeed found to give cultures containing a small proportion of bacteria sensitive to the F-specific phage MS2, and the proportion was increased in HFT preparations (148, 149). Electron microscopy showed that these *fi*$^+$ factors determined a sex pilus resembling the F pilus, and that the incidence of piliated bacteria in different HFT cultures was correlated with the frequency of resistance transfer and of cells sensitive to phage MS2 (57). Among different wild-type R factors, only those which were *fi*$^+$ conferred sensitivity to F-specific phage (129, 149); thus, F and *fi*$^+$ R factors determine the synthesis of similar pili. At the same time, the functional relationship of these R factors to F, as originally defined by the *fi*$^+$ character, indicates that the pilus-producing genes in both factors are subject to the regulatory mechanism of the R factor. Because the structural gene for pilus synthesis in F as well as in R is repressed by an *fi*$^+$ factor, repression acts in the *trans* as well as in the *cis* position, and must therefore be effected by a cytoplasmic repressor (113).

Derepressed (drd) mutants. If regulation of pilus formation and conjugating ability is due to a repressor, mutant R factors should occur which fail to produce the repressor, and which therefore conjugate at high frequency. Such a mutant should give cultures like those of F$^+$ bacteria, cultures in which virtually every bacterium is piliated, is able to conjugate, and is sensitive to F-specific phage. Moreover, it should no longer suppress the function of F. Egawa and Hirota (62), while studying the action of R factors on F, transferred the *fi*$^+$ (or *i*$^+$) R factor R100 to an Hfr strain and isolated clones of bacteria which produced genetic recombinants at the frequency characteristic of the normal Hfr strain. These carried a mutant R factor which no longer suppressed the fertility of Hfr bacteria. Further examination of this mutant, R100-1 (166), showed that, besides losing its ability to repress F, the mutant no longer repressed itself. R100-1$^+$ F$^-$ bacteria conjugated at a much higher frequency than bacteria carrying wild-type R100 and could be seen in the electron microscope to produce pili closely resembling F pili. The cytoplasmic repressor produced by the *i* gene of R100 was therefore able to suppress its own pilus genes as well as those of F, showing that R100-1 was an *i*$^-$ constitutive mutant which failed to synthesize repressor, as distinct from an *o*^{c} constitutive mutant insensitive to repressor (113). Other derepressed mutants were isolated from another R factor, R1, by Meynell and Datta (151), not by selecting for loss of activity against F, but by looking directly for variants which transferred drug resistance at high frequency. With these mutants of R1, conjugation, as measured by transfer of drug resistance, occurred at 300 times the frequency observed with the wild type; R$^+$F$^-$ cultures were lysed macroscopically by the F-specific phage MS2 with an efficiency of plating about 0.2 of that on an F$^+$ strain; and 50 to 90% of the bacteria could be seen in the electron microscope to produce pili able to adsorb phage MS2.

Derepression could be due either to absence of repressor or to insensitivity to repression, analogous to the *i*$^-$ and *o*^{c} mutations, respectively, in the lactose regulatory system (113); these possibilities can be distinguished by testing the effect of the mutant R factor on F and by examining the effect of a second, wild-type, R factor on the mutant. R100-1 was an *i*$^-$ mutant, since it no longer repressed F, and R1*drd* was shown to be of similar kind, for it remained susceptible to the

repressor produced by a second *fi*+ R factor introduced into the same cell.

fi− *Factors*

The *fi*− R factors do not suppress the function of F and do not determine the production of an F-like pilus. Their lack of effect on F could mean either that they made no repressor (like *i*− mutants of an *fi*+ factor) or that they made a repressor inhibiting their own function which had no effect on F. If the former were the case, the efficiency of transmission of *fi*− factors would always be high and could not be further increased when the factor had just entered a new host. In general, however, *fi*− factors are transferred by established cultures at no higher frequency than *fi*+, and HFT systems can be produced with as high a proportion of *fi*− R factors as of *fi*+. Conjugation is therefore repressed with at least some *fi*− factors, just as with the *fi*+ class. Conjugating ability might also be limited by defective function, as observed with defective mutants of F (42, 79). The *fi*+ factors are manifestly repressed since they repress F, but, although repression also occurs with many *fi*− factors, there is nothing at present to prove that others which conjugate at low frequency may not be defective. Any repressed *fi*− factor should yield derepressed mutants analogous to the derepressed mutants of *fi*+ R factors, and such mutants have indeed been isolated by selecting clones of R^+ bacteria which transferred drug resistance at greatly increased frequency (151). With these mutants, nearly 100% of the bacteria transmitted their drug resistance in 10 min, but, although virtually every bacterium was expressing its conjugating function, the cultures remained insensitive to F-specific phage. The surface structure in these conjugating bacteria was therefore not the F pilus. The nature of the structure became clear once cultures were examined by electron microscopy (Fig. 2), for sex pili were seen that were morphologically distinct from F pili but which bore a close resemblance to the I pilus already identified in HFT cultures carrying ColIb-P9 (152). Thus, the sex factor of the *fi*− class of R factors appeared to be I. The presence of the I pilus in *fi*− cultures was confirmed by the isolation of the filamentous I phage, using a derepressed *fi*− R factor as indicator (G. G. Meynell, Proc. European Phage Meeting, 1967, p. 14); this phage lysed cultures with derepressed *fi*−, but not *fi*+, R factors and, in addition, grew in HFT preparations of ColIa and ColIb.

OTHER TRANSMISSIBLE PLASMIDS

The various transmissible plasmids recognized in the *Enterobacteriaceae* made their first appearance associated with bacterial characters that were found to be passed from cell to cell in mixed culture. Each of these plasmids must therefore possess its own sex factor, and, since this is linked to an associated character, they are analogous to F′ derivatives of F (110). Prominent among such transmissible plasmids are the Col factors, notably ColV, ColB, and ColI (75). There are also several other isolates of various kinds: the F_0-*lac* factor was originally detected in a strain of *Salmonella typhi* observed to ferment lactose (15) and P-*lac* was detected in the same way in a strain of *Proteus mirabilis* (69). A surface antigen, the K88 antigen, composed of a protein with hemagglutinating activity (173, 204, 205) present in strains of *E. coli* causing enteritis in pigs, is determined by a plasmid with sex factor activity (172, 202); a soluble hemolysin commonly found in strains of *E. coli* pathogenic for pigs, as well as an enterotoxin causing edema of the pig gut, are also determined by extrachromosomal transmissible genetic elements, known respectively as Hly (202) and Ent (H. W. Smith, J. Gen. Microbiol., *in press*).

Col Factors

Among the Col factors which are transmissible, and which thus comprise sex factors, are ColV-K30; ColV-K94 [also found to determine production of an I colicin, so that it should perhaps be designated ColV,I-K94 (161, 162)]; ColV,B-K260; ColB-K77; ColB-K98; ColIb-P9 [as well as 20 similar factors from wild strains of *S. typhimurium* (177)]; ColIa-CA53 (209); ColEla (136); ColEl-K30, transmissible in *S. typhimurium* (136, 177) but not in *E. coli* (35, 117); and determinants found in a variety of wild strains of enterobacteria for the production of colicins B, E, I, K, and V (74). The factors ColV-K30 (117, 141), ColV-K94 (117, 118, 141, 163), and ColV,B-K260 (77) have so many of the characteristics of F that they might result from an association between F and the colicin determinants (76), but the others appeared superficially to be sex factors of a different kind. However, the study of R factors has shown that sex factors may be closely related to F even when they exhibit few of the characteristics of F itself. The relationship is exposed by the phenomenon of F-inhibition (the *fi* character); Col factors with sex factor activity can also be classified as *fi*+ or F-like, on the one hand, or as *fi*− and unrelated to F, on the other. ColB is F-like because ColB-K98 is *fi*+, as it suppresses the fertility of F^+ and Hfr strains (180), and F^- cultures carrying ColB-K77 or ColB-K166 propagate the F-specific phage, MS2, and evidently contain a proportion of cells producing an F-like pilus (150). Increase of phage

MS2 is also observed in many other, but not all, colicinogenic strains (150). The rate of transfer of ColV-K94 from a Col^+ to a Col^- population is equal to that of F, and that of ColV-K30 is very nearly as high. Also, the F-specific RNA phage μ_2 produces plaques on K-12 strains carrying ColV-K94 and ColV-K30 with an efficiency of plating of 1 and 0.2, respectively (141). Bacteria carrying ColV-K94 produce pili to which both isometric and filamentous F-specific phages attach (29). Thus, these ColV and ColB factors carry the gene determining production of an F-like sex pilus, but, whereas ColV, like F, is derepressed, ColB resembles *fi*$^+$ R factors in producing a repressor regulating the activity of the genes for pilus formation in F as well as in itself.

ColIb-P9, ColIa-CA53, and ColEl-K30 do not repress F (35, 163, 164) and, although they can act as sex factors (136, 159, 163, 177), do not confer sensitivity to F phages (129, 150). They are thus *fi*$^-$. ColIb-P9 constitutes the prototype for the class of I-like sex factors and its pilus was the first to be identified (152).

ColEla is also associated with an I-like sex pilus (Fig. 6; 153). When derepressed mutants were isolated, it was clear that the determinants for colicin production and the I-like sex factor were unlinked, just as ColE2 and ColI may coexist in one strain. In the case of ColEla, however, its accompanying I-like sex factor did not determine colicinogeny or any other incidental character, and was thus comparable to F (S. Edwards and G. G. Meynell, *unpublished data*).

Other Plasmids

The sex factors associated with other transmissible characters can be tested for their *fi* character in the same way as those associated with antibiotic resistance or colicin production, as well as for their ability to determine the production of an F-like or I-like sex pilus. The Hly and Ent factors, responsible for production of a soluble hemolysin and an enterotoxin, respectively, and at least one factor determining the K88 antigen, all repress F (202), and Hly and Ent also determine the receptor for F-specific phage (129). F_0-*lac* appeared in a lactose-fermenting strain of *S. typhi*, which transmitted this character to other members of the *Enterobacteriaceae* (65). F^+ cultures carrying F_0-*lac* are still lysed by F-specific phage, but, although F_0-*lac* itself determines the synthesis of a pilus (22) which can be seen on up to 50% of the bacteria (129), the cultures are not lysed by isometric F phage (65). This does not mean that the F_0-*lac* pilus is entirely different from the F pilus, however, for cultures are lysed by the F-specific filamentous phage M13. Electron microscopy shows that the isometric phage does not attach to the side of the pilus, although the filamentous phage adsorbs to the tip (129).

Lederberg, Cavalli, and Lederberg (132) reported that 40 of 2,000 wild strains of *E. coli* showed some degree of fertility with a K-12 F^- recipient, and some donated an agent which converted the recipient into a genetic donor. Two of these strains, WG3 and WG4, were later respectively shown by Bernstein (18) to restore fertility to, or give recombinants with, the defective F^+ strain 58.161/*sp* (96, 150) and an F^- strain. Ørskov and Ørskov (171) found that 5 of 275 wild strains of *E. coli* gave recombinants with F^- bacteria; Furness and Rowley (78) likewise described eight strains of *E. coli* which converted either strain 58.161/*sp* or an F^- strain to fertility after growth in mixed culture. Bacteria sensitive to F-specific phage MS2 were detected in strains WG3 and WG4 as well as in four of the latter strains (150), which thus evidently carried sex factors related to F. Anderson and Lewis (10) isolated a sex factor from strains of *S. typhimurium* whose presence they detected by a change in phage type. Like other sex factors such as F and ColI, it brings about the transfer of plasmids which are in themselves nontransmissible.

RÉSUMÉ

The relationship between different sex factors can therefore be assessed by, first, the specificity of the mechanisms controlling synthesis of the sex pilus, and, second, the properties of the sex pili themselves as revealed by their morphology, capacity to adsorb F and I phages, and antigenic structure (Tables 2 and 3). Other criteria are described in subsequent sections, but this is a convenient point at which to summarize the results directly relating to the pilus.

TABLE 3. *Reactions of sex pili with donor-specific phages and with antiserum*[a]

Pilus	Phage		Antiserum to			
	F	I	F	ColV	R1	R144
F	+	−	+	+	+	−
ColV	+	−	+	+		
F-*lac*	+	−	+		+	−
F_0-*lac*	+	−	+			
R1 (*fi*$^+$)	+	−	+	+	+	−
ColIb-P9	−	+	−		−	+
R64 (*fi*$^-$)	−	+	−			+
R144 (*fi*$^-$)	−	+	−		−	+

[a] Minus signs mean that no antibody was seen on pili when serum was used at a concentration considerably higher than that giving a + reaction with homologous pili.

It will be appreciated that, unless sex pili are produced by the majority of the bacteria, the culture will fail to plate sex phages, since any potential plaques will be overgrown by non-piliated cells. Derepressed cultures, either de-repressed mutants or HFT preparations of wild-type repressed plasmids, are also needed for electron microscopy. Although the antigens of the sex pili determined by F and the closely related R100-1 have been examined by agglutination tests (100, 192, 193), following the pioneer work of Ørskov and Ørskov on the F pilus (170), direct observation of the union of antibody and pilus by electron microscopy (106, 128, 129) has considerable advantages (Fig. 7, 8). The specificity of the reaction is evident immediately, antisera no longer need to be absorbed, and the tests can be performed on cultures which agglutinate spontaneously, a serious difficulty encountered with many derepressed mutants in conventional agglutination tests (100, 129, 151). Furthermore, the tests require little antiserum since they can be performed entirely on the microscope grid (128). Fewer factors have yet been analyzed by serological means than with phage, but the relationships apparent in Table 3 are consistent with those deduced from the other tests.

Repressed cultures can nevertheless be examined, by looking for an increase in titer following addition of F-specific or I-specific phage. Some 60 plasmids have been tested, all of independent origin so far as is known (129). Their bacterial hosts fell into four classes (Table 4), of which the first two corresponded to the presence of typical F-like and I-like factors, respectively. The first (F-like) contained a majority of fi^+ R factors, no fi^- factors, and 10 other plasmids: F, F_0-*lac*, ColV-K94, ColV-K30, ColB-K77, Hly, Ent, K88, and the sex factors of strains WG3 and WG4 (18). The second (I-like) contained 20 fi^- R factors and 6 other plasmids: ColIa-CA53 -CT2, ColIb-P9, -CT4, and the I-like sex factor accompanying ColEla-16, -18. It also contained one fi^+ R factor, R62. The third class of cultures propagated both F and I phages, and carried both an F-like and an I-like factor; probably, the strain carrying R62 really belongs to this class and carries an I-like factor in addition to one which is fi^+ but does not give an increase of F phage. The fourth class propagated neither F nor I phages. Here (as well as in the case of the fi^+ R62), the sex factors may have been of other types, but the behavior of these cultures is also explicable by severe repression, so that too few pili were formed for an increase of phage to be detectable, or by the formation of a variant pilus. The effect of repression is illustrated by R64, for the behavior of derepressed R64 shows this factor to be I-like but its nature is not evident from tests made with repressed wild-type cultures, for so few pili are produced that no increase can be detected. An example of a variant pilus is provided by F_0-*lac*, whose pilus is F-like in appearance, antigenic structure, and adsorption of the filamentous phage, M13, yet fails to adsorb the isometric phage MS2 (65, 129). Minor differences in pilus structure are reflected by a drop in efficiency of plating of isometric F phage and by heterogeneity in plaque diameter, in contrast to a reduction in the proportion of piliated cells which quickly abolishes plaque formation entirely (150). A difference between the pili determined by F and by R100-1 was detected in this way, in agreement with the electron microscopical observation that fewer phage particles attached to R100-1 pili (166). It may be that the pili of ColV-K30 also differ slightly from those of F, for, here too, F phage plated less efficiently on the Col^+ culture (141).

In general, however, RNA phages such as the isometric phage MS2 are greatly to be preferred in these experiments to DNA phages like the filamentous F or I phages, which are liable to restriction (13) imposed by many R factors (S. Austin, *in preparation*). Unfortunately, no RNA I phages have yet been isolated, and failure of a strain to propagate the filamentous I phage might therefore be due to restriction, not to absence of the I pilus.

To use sex pili for identifying sex factors in different bacterial strains is to assume that the structure of the pilus is unaffected by the host. All the present evidence, and also that derived from the study of a number of plasmid-determined enzymes (32, 50, 68, 196), suggests that the protein determined by a given gene is the same, regardless of the bacterial species. Yet, it should be mentioned that Sekijima and Iseki (192) found minor differences in the F pili formed by *Salmonella paratyphi* B and *E. coli*, using agglutination tests with cross-absorbed sera; they also observed the same phenomenon with R100-1 (193), although it is not clear how spontaneous agglutination of the cultures (100) was overcome. The host may certainly affect the expression of plasmid genes: those determining β-galactosidase formation in F_0-*lac* (68) and P-*lac* (232) are not regulated as efficiently in *Proteus* as in *E. coli*; in addition, F^+cultures of different species or strains, or even different lines of *E. coli* K-12, differ widely in the extent to which pili are formed, as shown by differences in donor ability (235) and in sensitivity to F-phage (39, 53, 54).

With the exception of F and ColV, all wild-type factors appear to be repressed, and there are two reasons which might account for this. First,

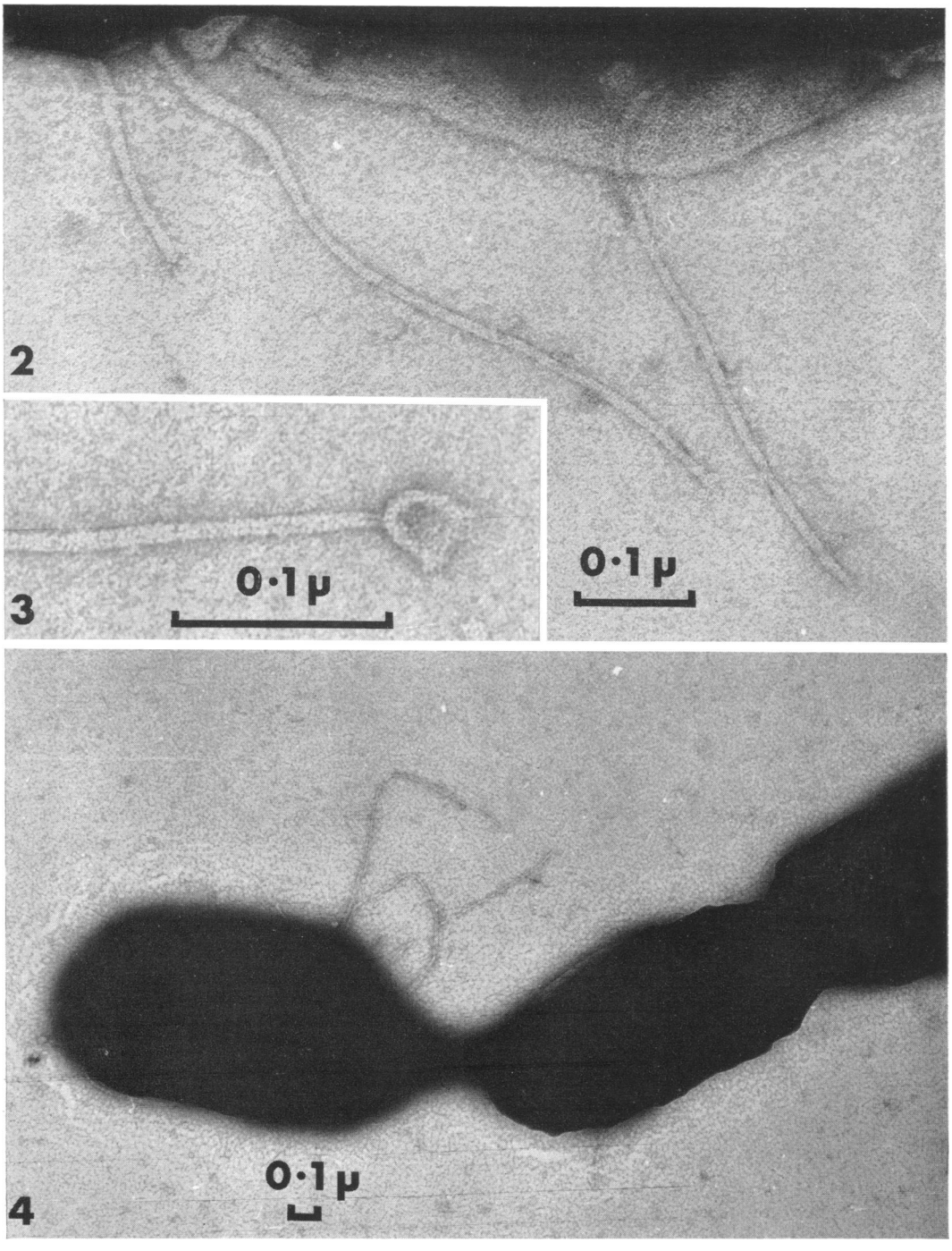
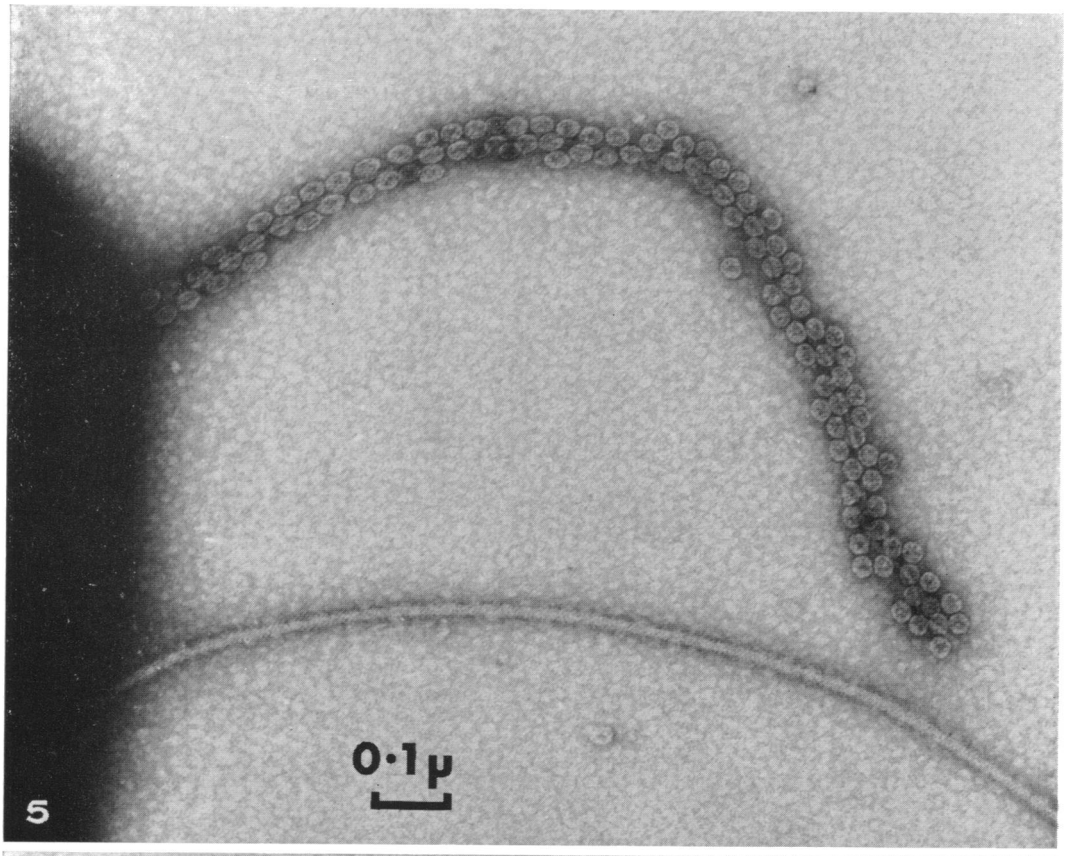


FIG. 2. *I-like sex pili determined by the derepressed fi⁻ R factor, 144-3.*
FIG. 3. *Terminal knob on an F-like sex pilus determined by R1.*
FIG. 4. *Mutant of Salmonella typhimurium unable to form flagella or common pili, with two clusters of I pili determined by ColIb-P9.*



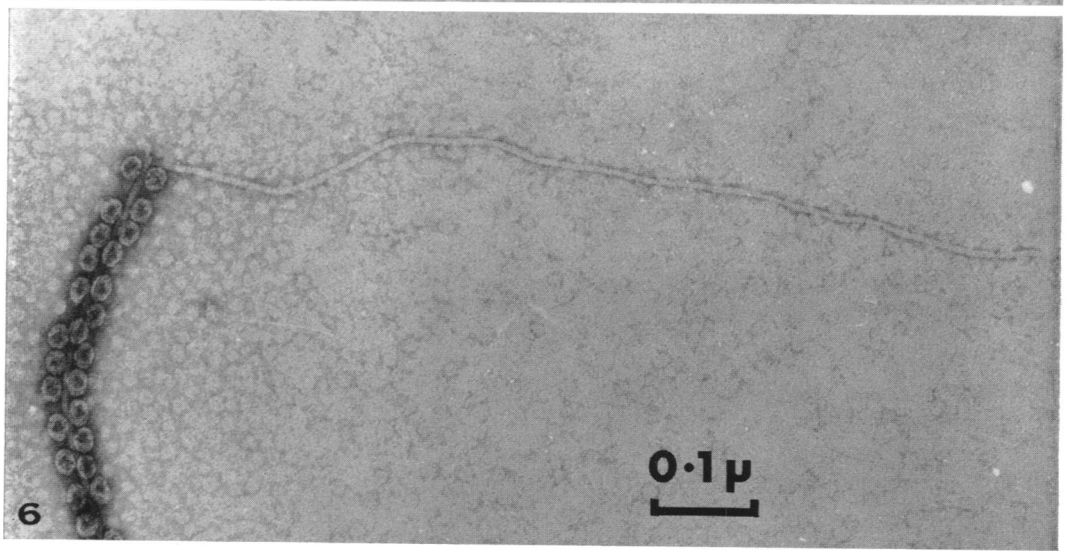


FIG. 5. *F pilus of F-lac, heavily labeled with phage MS2, with a flagellum.*

FIG. 6. *F pilus determined by F-lac, heavily labeled with phage MS2 and showing the filamentous phage, M13, adsorbed tip to tip to the sex pilus (29).*

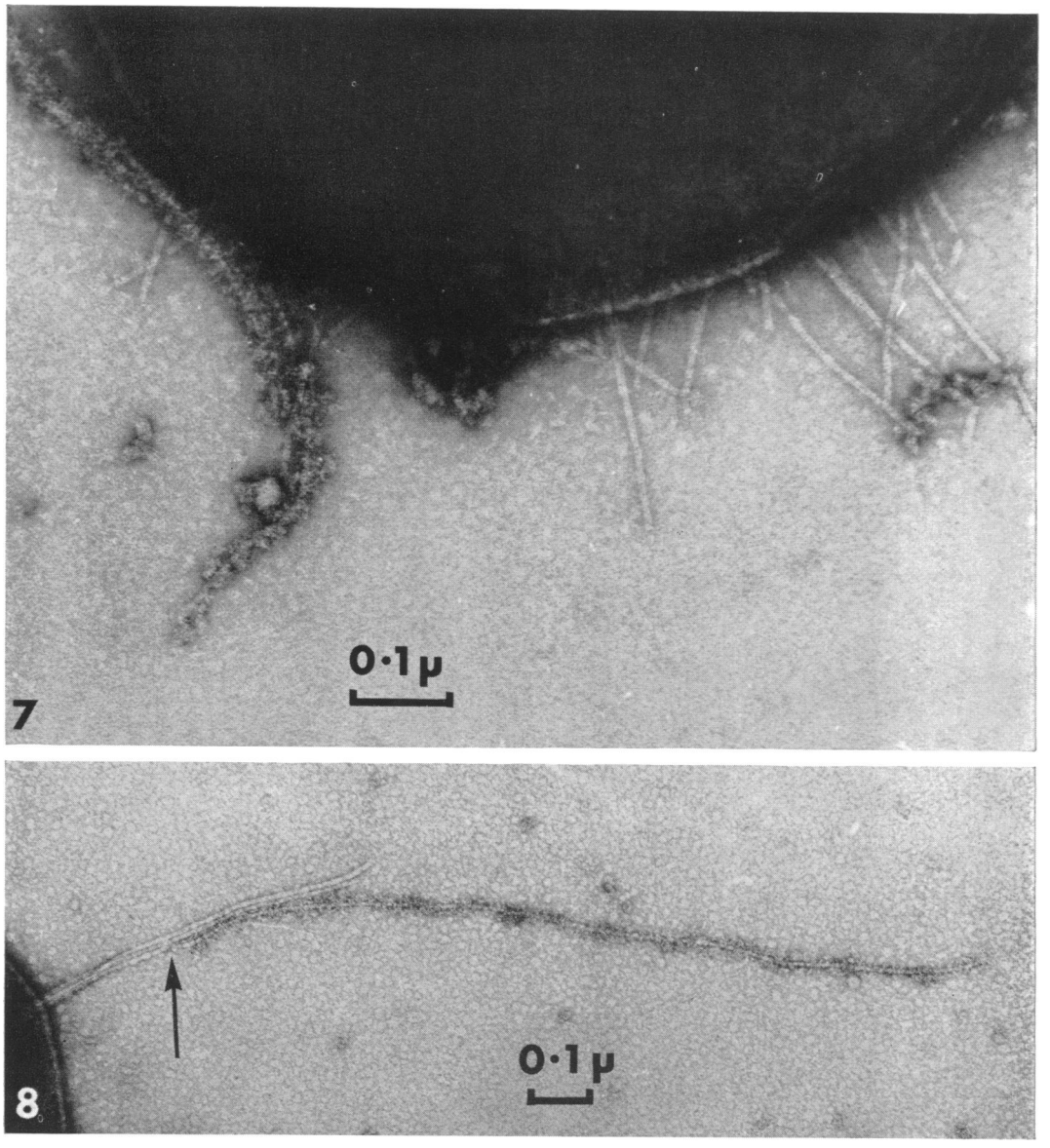


FIG. 7. *Cell from an HFT preparation of ColE1a-16 after exposure to antiserum for the fi⁻ R factor, 144. On the right are a number of common pili; on the left are several I-like sex pili coated with antibody. Preparation negatively stained with uranyl acetate (128).*

FIG. 8. *Three I-like sex pili, one of which has adsorbed the filamentous I phage, If1. The phage is labeled with antibody (128), and its junction with the pilus is indicated by the arrow.*

strains carrying derepressed mutants of R factors or of I grow noticeably more slowly than those with the wild-type plasmid, just as do strains fully induced for an enzyme like β-galactosidase (167, 178), possibly because a significant fraction of the cell's syntheses is diverted to a product irrelevant to growth. This effect is not apparent, however, with F and ColV, whose cultures grow as fast as those of F⁻ Col⁻ strains. Second, repressed sex factors are likely to be the more abundant because the majority of cells produce no sex pili and are therefore resistant to donor-specific phages. The abundance of these phages is in turn explained by the relatively high incidence of repressed sex fac-

TABLE 4. *Ability of cultures carrying various transmissible plasmids to propagate F and I phages*

Phage propagated		Cultures carrying		
F phage	I phage	R factor		Col factors and other transmissible plasmids
		fi+	*fi*−	
+	−	11[a]	0	10[g]
−	+	1[b]	20[e]	6[h]
+	+	5[c]	0	0
−	−	2[d]	6[f]	0

[a] R1, 28, 36, 51, 52, 82, 100-1*drd*, 124, 136, 192*drd*, 312.
[b] R62.
[c] R2, 73, 77, 114, 196.
[d] R27, 128.
[e] R39, 56, 64*drd*, 92, 342*drd*, 143, 144*drd*, 145, 163, 183, 293, 296, 297, 298, 299, 302, 306, 307, 308, 313.
[f] R45, 46, 199, 300, 305, 310.
[g] F, F_0-*lac*, H1y, Ent, WG3, WG4, ColV-K94, -K30, ColB-K77, -K166.
[h] ColIa-CA53, -CT2, ColIb-P9, -CT4, ColE1a-16, -18.

TABLE 5. *Incidence of fi+ and fi− R factors in 206 strains of Salmonella and Escherichia coli isolated from natural sources*

Strains	Series[a]	*fi*+	*fi*−	Total
		%	%	
Salmonella	1	30	70	83
	2	35	65	34
E. coli	1	80	20	50
	2	74	26	39

[a] Series 1 was isolated in England (Datta, *unpublished data*), and series 2 in Italy (E. Romero, *personal communication*).

tors which give a continuous supply of piliated bacteria to support phage growth.

The relative incidence of *fi*+ and *fi*− R factors is different in naturally occurring strains of *Escherichia* and *Salmonella*. Table 5 shows that *fi*+ factors are found more commonly in *E. coli*; *fi*− factors predominate in *S. typhimurium*, which agrees with the observation that, although many different Col factors, F-like as well as I-like, are present in *E. coli* (86), ColI and ColE are almost the only ones found in *Salmonella* (87). This may be due to differing stability in the two hosts, for, in the laboratory, F is more readily lost from *Salmonella* than from *E. coli* (E. Meynell, *unpublished data*), and ColK and ColB are unstable in *Salmonella*, whereas ColI, ColEl, and ColE2 are stable (177). The overall incidence of *fi*− R factors is higher than appears in Table 5, for 5 to 10% of strains carrying an *fi*+ factor also carry one which is *fi*− and whose presence is masked when the *fi* character of the strain is tested (129; Romero and Meynell, *in preparation*). Tanaka et al. (213) also found a high proportion of strains with two R factors, probably *fi*+ and *fi*−.

OTHER CRITERIA FOR CLASSIFICATION

The degrees of relationship between plasmids can be further assessed by a variety of means, which have so far been applied to only a limited extent. The most direct is a comparison of their DNA base ratios. Another is to determine whether pairs of plasmids exhibit superinfection immunity, as shown by their inability to coexist stably in the same strain. A different form of interaction which can be examined is the ability of one sex factor to restore the function of another that is defective, whether autonomous or integrated, by recombination of their genomes or by complementation. The actual patterns of recombination between plasmid and host chromosome, or between plasmid and plasmid, serve to demonstrate regions of genetic homology and, hence, relationship.

Base Ratios

Biophysical studies show that R factors do not differ greatly in size from F or the smallest F′ factors, having a molecular weight of 2×10^7, equivalent to about 1% of the chromosome of *E. coli* (56, 66, 67, 145, 186, 219). Isopycnic density gradient centrifugation in CsCl indicates that nine-tenths of the DNA of F has 50% guanine-cytosine (GC), like the chromosome of *Escherichia*, *Shigella*, and *Salmonella*, whereas the remaining one-tenth has 44% GC (66, 186). All R factors examined contain some DNA with 50 to 52% GC, pointing to an evolutionary relationship (186), but the size of this fraction differs enormously from one factor to another. Thus, R4 of Mitsuhashi contains equal amounts of 58 and 52% GC, whereas Watanabe's R222 has 85 and 15% of these components, respectively (186). The loss of resistance genes by certain R factors is accompanied by changes in density which indicate that the gene determining tetracycline resistance is 50% GC, whereas those for resistance either to chloramphenicol alone, or to a complex of sulfonamide, streptomycin, and chloramphenicol, consist of DNA with 56% GC, similar to the chromosome of such species as *Aerobacter*, *Klebsiella*, or *Serratia*. The presence of 56% GC DNA appeared to be correlated with chloramphenicol resistance in a series of different wild-

type R factors (67), and, since it is universally recognized that chloramphenicol resistance is present only on *fi*$^+$ (or F-like) R factors, it was perhaps surprising that it should be found only with a DNA so unlike that of F. However, the correlation broke down in the case of one R factor, for all its known functions, including chloramphenicol resistance, were retained after loss of the 56% GC fraction (186). Few analyses of DNA have been made for Col factors. ColI and ColE1 are known to be of comparable size; ColE2 is smaller (197) and ColE1 consists of DNA of the same density as the chromosome of *E. coli* (231). P-*lac* consists, either wholly or in large part, of 50% GC DNA, but, unlike F (66), it shows no hybridization with *E. coli* DNA (232).

The great difference in base composition of individual R factors shows clearly that GC content cannot be correlated in a simple fashion with, for example, the *fi* character of the plasmid. The heterogeneity may, indeed, be the outcome of multiple origins and of repeated genetic recombination between factors carried by the same cell, as suggested by Falkow (67), for recombination can readily be observed in the laboratory.

Superinfection Immunity

The most obvious example of this phenomenon is the immunity shown by a lysogenic strain to infection by phages related to its prophage. With phage λ or P2, the superinfecting phage enters the immune cell but, being unable to replicate, is diluted out during bacterial multiplication (19, 109); in addition, the behavior of phage P22 shows that the superinfecting phage genes may disappear and are said to be excluded (218). Both exclusion and suppression of multiplication occur with transmissible plasmids that are related (41, 61, 189). This is most clearly seen when an F-*lac*$^+$ donor is crossed with a *lac*$^-$ F$^+$ (or Hfr) recipient, when the fraction of recipients expressing *lac*$^+$ is only 1 to 2% of that observed with an F$^-$ recipient. Moreover, the *lac*$^+$ gene is not continuously replicated in the vast majority of the F$^+$ cells which express it, since the colonies on lactose indicator medium are sectored instead of being homogeneously *lac*$^+$ (189). Although exclusion can be transiently overcome by starving an F$^+$ recipient to produce an "F$^-$ phenocopy" (132), its cause is at present unknown. It is not due to repulsion between the F pili of donor and recipient since F pili are often absent; moreover, the same phenomenon occurs in crosses with an LFT ColI recipient culture (203; G. G. Meynell, *unpublished data*) in which I pili are formed by less than 0.1% of the cells (152). A possible explanation is that the recipient normally contributes a function essential to an early stage in transfer which is repressed if it carries the plasmid, and which is derepressed nonspecifically by starvation in the same way as starvation removes the ability of a strain to restrict unmodified DNA (135, 137).

The subsequent failure of inheritance of F-*lac* in an F$^+$ recipient which leads to sectored colonies can be interpreted by the "replicon model" (111, 112). This postulates that genes, in order to replicate, must be part of a genetic unit, the replicon, which occupies a unique intracellular specific site where replication can be initiated, probably on the cell membrane. Different kinds of plasmid have different sites and therefore do not interfere with each other's continued existence within the cell. There appears to be only one copy of F per chromosome, judging from (i) a comparison of the relative amounts of F and chromosomal DNA, with the amount of DNA in a single copy of the F factor (56, 66, 67, 145, 186); (ii) the level of β-galactosidase synthesis, which is approximately doubled when a *lac*$^+$ cell also carries F-*lac*$^+$ (112); and (iii) the manner in which thermosensitive F-*lac*$^+$ disappears from a culture growing at a temperature sufficient to inhibit its replication (46).

The relationship between different plasmids can therefore be assessed by superinfection experiments, though it will be seen that, if the donated plasmid does not determine an easily recognized character, like *lac*$^+$, and is not stably expressed by the recipient, it may be impossible to know whether immunity results from exclusion or from inhibition of replication.

No R factor, whether *fi*$^+$ or *fi*$^-$, has yet been described that cannot exist stably with F. The difference in frequency of transfer to F$^+$ and F$^-$ recipients is only two- to fivefold (222, 224). Thus, despite the relationship between F and the sex factors of *fi*$^+$ R factors indicated by cross-repression and the structure of their sex pili, these plasmids do not exhibit superinfection immunity. On the other hand, immunity is evident with most pairs of wild-type R factors when both members are *fi*$^+$ or *fi*$^-$ (156, 227; E. Meynell, *unpublished data*). Similarly, R factors conferring resistance to several drugs yield segregants lacking one or more of the resistances (158, 226), and each segregant of this kind which retains sex factor activity lowers the ability of the host to accept the other (224). When a particular pair of R factors behaves in this way, both may nevertheless coexist unstably if the medium contains a combination of the drugs selective for each (155, 156, 227). Under these conditions, both factors are probably maintained simultaneously by constant reinfection within the culture; if one or both drugs are omitted, segregant bacteria immediately emerge, carrying only one of the factors. Ulti-

mately, stable cultures resistant to all the drugs appear in which all the resistance genes are evidently present in a single recombinant factor, for, whenever this possibility has been tested, all the resistances are transduced as one physical unit by phage P1 (156, 227). Recombination of this kind is reported to occur between two fi^+, but not between an fi^+ and an fi^- factor (223, 227); however, this may mean no more than that recombination could not be detected with the latter because these factors can coexist without inhibition, so that there is no selection for the emergence of a recombinant.

The sex factor of ColV, which resembles F in occurring naturally in the derepressed state, also shows itself to be closely related to F on the basis of superinfection immunity (117, 118). F excludes ColV3-K30, and ColV2-K94 excludes F from an F^+ but not from an Hfr strain (141) in which F is integrated with the bacterial chromosome. With certain combinations of F and ColV, a fraction of bacteria emerge which carry both factors stably (41, 61, 141), but it is not yet certain that the bacterium and factors are not variants of the original.

The data concerning other transmissible plasmids are not extensive. Transfer of F_0-*lac* to F^+ or Hfr cells is reduced about 100-fold (65), but, once it is accepted, both factors can evidently be stably carried by the cell. F and ColI can coexist stably together (159). There are two forms of ColI factor, ColIa and ColIb (206), each of which confers resistance to superinfection with the other; a doubly colicinogenic IaIb clone has never been obtained. Judged by the rate of transfer of the nontransmissible plasmid, ColE2, from an HFT ColIa culture to a ColIb recipient, the frequency of conjugation is about 0.001% of that with a Col^- recipient (G. G. Meynell, *unpublished data*). The rate of transfer of ColE1a, mediated by an I-like sex factor, is similarly lessened if the recipient carries ColI, and vice versa (153). ColX and ColK can coexist, but either is eliminated from a proportion of bacteria that receive certain R factors (120).

A considerable number of plasmids can be carried simultaneously, provided they are not of a kind which specifically interfere with one another. For example, Dubnau and Stocker (58) described a culture of *S. typhimurium* carrying F (integrated in the chromosome), ColIb-P9, ColE1-K30, ColE2-P9, and R2; cultures have been obtained with Hly, Ent, K88, and R factors (H. W. Smith, J. Gen. Microbiol., *in press*) and with an R factor, two Col factors, and a surface antigen factor (92).

When the chromosome and F in an F^+ culture are distributed at cell division, either the old or the new copies of each are jointly inherited by the same daughter cell, providing direct evidence for a single "unit of transmission," which is likely to be the region of the cell membrane containing the replication sites for the two individual replicons. Extension of the cell membrane during growth would, therefore, apportion the copies between the daughter cells (47, 112), There is nothing to suggest any fundamental difference between one sex factor and another in the way in which they ensure their inheritance, and thus it is probable that this unit of transmission can carry more replicating structures than F and the chromosome. Nevertheless, the membrane does not appear to have an unlimited capacity to accommodate different replicons, judging from the relatively few combinations of different plasmids which readily coexist in the same cell.

Restriction. In addition to the exclusion associated with superinfection immunity, the fate of foreign DNA donated to a plasmid-carrying strain is subject to restriction (12). The particular case of restriction of phage DNA is well known in connection with strains lysogenized by temperate phage, but it is found equally commonly with those carrying plasmids like F(53, 54, 85, 99, 142, 191, 236), Col factors (209, 229), and many R factors (14, 195, 228, 230, 234). This is of considerable clinical importance in the phage-typing of *Salmonella* (9, 71) in which the diagnostic patterns of sensitivity and resistance are largely due to phage- or plasmid-determined restrictions and may therefore be altered if the test strain has acquired an R factor or some other transmissible plasmid (6, 11, 82, 83). Tests for restriction of a number of phages by different R factors show that, although there is a tendency for certain combinations to be affected together, the patterns of restriction are in general different from one R factor to another and are not particularly associated with whether the R factor is F-like or I-like. Table 6, taken from unpublished results kindly provided by D. Bannister, illustrates this point. Little is known of how the restrictions are brought about, or whether the mechanism is the same as with the r^+ gene of the bacterium (38), although Watanabe et al. (211, 230) showed that, with certain R factors, the phage DNA penetrates the cell only to be broken down.

Genetic Interactions Leading to Chromosomal Transfer

R factors (210) and other plasmids like ColI (175) and K88 (172) bring about gene transfer leading to recombination between F^- bacteria. Current models for chromosome transfer are derived almost entirely from experiments with the F factor, and transfer is generally believed to depend on integration of the sex factor with the

TABLE 6. *Phage restrictions by R factors*[a]

Phage								No. of R factors	
λ	φ80	P1	P2	φ+	T3	BF23	W31	*fi*+	*fi*−
+	+	+	+	−	−	−	−	0	1
+	+	+	−	−	−	−	−	3	3
+	+	−	+	−	−	−	−	1	0
+	+	−	−	−	−	−	−	4	1
+	−	−	−	−	−	−	−	1	1
−	−	−	−	−	−	+	−	2	1
−	−	−	−	−	−	−	+	2	1
−	−	+	−	−	−	−	+	1	0
−	−	−	−	+	−	−	+	4	12
−	−	−	−	+	+	−	+	1	2
−	−	−	−	−	−	+	+	0	1
−	−	−	−	+	−	+	+	2	2
+	−	−	−	+	+	+	+	0	3
−	−	−	−	−	−	−	−		100

[a] Restriction was recorded as + when the efficiency of plating of the phage was 10^{-2} or less of that on the R− host. Sources of phages: φ80 (147), φ(54), BF23 (209), and W31 (229).

chromosome, following a crossover between the two circular genomes of bacterium and F (2, 27, 28, 115, 190, 215), and preceded by pairing dependent on a homology in structure (66). Since F, or many temperate phages, can integrate at only relatively few points in the bacterial chromosome, integration exhibits specificity. Relationships between different plasmids may therefore be revealed by their relative proneness to integration and by the sites at which integration can occur, as reflected by the recombination rate and by the incidence of particular recombinant classes, respectively.

When comparing recombination rates, it should be remembered that these are necessarily determined in part by the rate of conjugation. A distinction has often been drawn between R factors and ColI, with which recombination is infrequent [less than 10^{-7} per donor cell (210)], and F, for which the rates are far higher [10^{-4} to 10^{-5} per donor cell (2)]. However, with F or ColV, almost every bacterium of the donor strain can conjugate, whereas other sex factors are repressed and conjugation occurs with only 10^{-2} to 10^{-4} of the cells at any one time. Other things being equal, when conjugation itself is infrequent, the numbers of genetic recombinants will be correspondingly small. To detect specific differences in chromosome transfer, recombination rates should be standardized in terms of the rates of conjugation. This is difficult to do precisely with wild-type R or Col factors because recombination rates are so small, but can readily be done with factors that are derepressed, either naturally or following mutation.

The interpretation of recombination experiments is based almost entirely on analyses of F+ and Hfr matings. In an Hfr culture, integration of the sex factor is stable, whereas the donor ability of an F+ culture results from occasional integration of the sex factor to produce a variety of Hfr variants in the donor culture (114). Although not all recombinants produced by an F+ culture are derived from stable Hfr variants of long standing (181), chromosome transfer has never been shown to occur in any way other than by physical continuity between chromosome and sex factor. Ten to fifteen homologous regions exist in the *E. coli* chromosome, judging from the number of stable Hfr variants that can be isolated with different polarities of transfer (25, 146).

Little is known of the degree of homology between R factors and the chromosome. Pearce and Meynell (J. Gen. Microbiol., *in press*) examined R1*drd*, an F-like R factor, and Meynell and Cooke (*in preparation*) examined four others, another F-like factor, R192*drd*, and three I-like factors, R64*drd*, R144*drd*, and R163*drd*. An F+ donor population gives approximately equal numbers of recombinants for all donor markers (233); the last four R factors were nearly as efficient as F in giving recombinants for various genes distributed around the chromosome (Table 7). However, R1*drd* was remarkable and provided direct evidence for integration in the same way as an F′ factor (2): it gave about as many recombinants as F for genes outside the *trp* region, but about 30 times more for *pyrF trp pur*B, which were transferred in ordered fashion from a fixed origin, exactly as by an Hfr strain. Chromosomal donors also transferred the R factor, just as bacteria carrying an F′ factor transfer the autonomous factor as well as the region of chromosome determined by the integrated F′ (2, 43). R1*drd* thus appears as a genetic element with particular affinity for a specific chromosomal site, which it probably acquired in the same way as an F′ factor, by recombination near the *trp* region of the chromosome of a previous host (1, 188). Crossing-over leading to insertion or release of a factor (190) occurs more frequently with F′ than with F, and the stability of F′ factors in either the integrated or the autonomous states is inversely related to the length of the homologous region provided by the incorporated segment of bacterial chromosome (2). R1*drd* alternates rapidly between integration and autonomy, suggesting a considerable length of R1*drd* to be homologous with the chromosome. Homology was associated with the sex factor rather than with the resistance genes, and it is of interest that R1*drd* integrated

TABLE 7. *Relative frequencies*[a] *of different classes of recombinant obtained with different sex factors (Meynell and Cooke, unpublished data)*

Donor strain	Autonomous sex factor	Recombinants		
		pro	*his*	*trp*
58.161	F	1 (1.8×10^{-5})	1 (10^{-5})	1 (1.6×10^{-5})
	R1*drd*	1	0.25	30
	R192*drd*	0.48	1	0.45
	R64*drd*	0.78	1	2.2
	R144*drd*	1	1	6.5
	R163*drd*	0.7	2	1.3
HfrC (F integrated near *pro*)	None	1 (5.5×10^{-2})	1 (10^{-5})	1 (2.3×10^{-5})
HfrCdr (defective F near *pro*)	None	5×10^{-5}	$<10^{-3}$	$<10^{-3}$
	R1*drd*	0.11	5	100
	R192*drd*	5.5×10^{-4}	1.7	1
	R64*drd*	0.15	6	4
	R144*drd*	1.3×10^{-3}	2.2	4
	R163*drd*	2.4×10^{-4}	1	1

[a] Frequencies are expressed in relation to F and to HfrC in the upper and lower parts of the table, respectively. The actual frequencies for F and HfrC are shown in parentheses.

at one of the chromosomal sites where F integrates to give the strain HfrB10 (25). No bacterial characters are known to be determined by genes in this part of the chromosome, and thus it was not possible to detect chromosomal genes incorporated in the autonomous R1, as has been possible with F′ factors like F-*lac* (110) or F-*gal* (101).

With R1, R192, R64, R144, and R163, chromosome transfer proceeded almost as readily as with F, once the limitation on conjugation was removed by the use of derepressed mutants. The same was also true of the derepressed R factor, R100-1, which gave chromosomal recombinants at 10^{-3} times the frequency with which the R factor itself was transferred (99); the ability of R100-1 to give chromosomal recombinants could not be separated by mutation from the ability to bring about conjugation (99).

The F-like col factor, ColV, is derepressed in its wild-type state and resembles F so closely that it would be expected to be associated with the same pattern of chromosomal transfer. However, testing only *pro* and *his*, Kahn and Helinski (117) found that ColV-K94 gave 40 to 65% as many *pro* recombinants as F but only 5 to 10% as many for *his*. Macfarren and Clowes (141) found an even greater difference, for, with both ColV2-K94 and ColV3-K30, the frequencies of recombinants for three well-separated genes, *pro*, *his*, and *trp*, were all of the order of 5×10^{-7} to 5×10^{-6}, instead of about 10^{-4} as with F.

Recombination with ColI has not yet been measured with the derepressed factor, but wild-type ColI gives genetic recombinants at a frequency of about 10^{-8} (175). If the donor also carries ColE1, the frequency is increased 10-fold in *S. typhimurium*, but not in *E. coli*. All donor markers are transferred with equal frequency, and there is at present no evidence that conjugation is the result of integration with the chromosome (37).

Recombination Between Plasmids and Ability to Effect Each Other's Transfer

Relationships between plasmids might be shown in two ways: by genetic recombination, or by functional similarity sufficient for one to make good a mutational defect in the other. In practice, it is often difficult to decide which mechanism is responsible for a positive effect, as immediately becomes clear on considering defective F or the co-transfer of otherwise nontransmissible plasmids, like ColE2.

Interactions between sex factors. F^+ or Hfr strains carrying defective F factors can be selected either by insensitivity to F phage or by failure to give genetic recombinants; both characters are simultaneously affected, as a result of failure to produce the F pilus (34, 42, 45, 79, 96, 99, 133, 150, 165, 199). Mutants have occasionally been mentioned in which genetic transfer is affected without loss of F phage sensitivity (112), or in which phage sensitivity is lost without loss of donor ability; the actual transfer of genetic material is affected in the first kind, but, in the

second, failure to show macroscopic lysis may simply reflect a very small reduction in the proportion of piliated bacteria (150) not sufficient to produce a noticeable decrease in genetic recombinants. With certain mutants, the phage cannot infect, although it can adsorb (198; Curtiss and Caro, *unpublished data*). Attempts to analyze the steps in pilus production are complicated by the difficulty of obtaining bacteria simultaneously carrying more than one F factor, but defective Hfr strains have had their function restored when it has been possible to infect them with a second F factor (44). Such tests are more readily made with combinations of F and R factors which can coexist in the same cell, and the F-like factor, R100-1, has been shown to restore fertility to defective F^+ or Hfr strains (99, 166) with production of F pili (which can be distinguished from R100-1 pili by their affinity for F-specific phage).

Similar defective mutants of an R factor can be isolated if it is first derepressed; defective mutants of R100-1 have been isolated by selecting for lessened ability either to bring about their own transfer or to give chromosomal recombinants (99). Certain pairs of these defective mutants of R100-1 restored function to each other, and most of them restored the function of defective F mutants (99, 166).

On the other hand, there is no restoration of F pilus production when a cell carrying a defective F mutant is infected with an *fi*$^-$ R factor; if the *fi*$^-$ R factor is itself derepressed, I pili can be seen, but not F pili.

Interactions between sex factors can be examined with Hfr strains defective in conjugation, which are of two kinds. The first results from a mutation in the integrated F factor, and can be selected, as just described, by resistance to F-specific phage. The second is formed during detachment of an F factor, when the crossover, instead of occurring between F and the adjoining chromosome, takes place within the F factor itself. After such a strain has lost the resulting F′ by segregation, its chromosome still possesses a fragment of F at the original site (1, 184). When F is reintroduced, the integrated remnant provides a region of homology (an *sfa* or "sex factor affinity" locus) at which F integrates preferentially to reconstitute an Hfr strain with the original polarity of transfer.

Different sex factors can be tested for their ability to restore transfer with the original polarity to nonfunctional Hfr strains of either kind, but there are at least three ways in which restoration could be brought about. If the original polarity were restored by any type of sex factor, whether F-like or I-like, the intact factor could simply be acting nonspecifically by providing a functional conjugation bridge, and the stimulus to transfer in the inserted F factor would then be independent of the nature of the link between donor and recipient. Second, a factor (most probably one belonging to the F-like group) might replace the defect in the integrated F factor, so that the latter once more brought about the production of its own F pilus and conjugation bridge. Lastly, the original polarity of transfer would be restored by any factor possessing sufficient homology with F to favor its own integration within the integrated F in preference to chromosomal sites elsewhere. If, as suggested for F, transfer is determined by attachment of the integrated sex factor near the base of the sex pilus (22, 47, 111, 112) and if union with the recipient stimulates the replicator of the sex factor to produce the free end of newly synthesised DNA which is donated (2, 81), the leading end, or *origin*, of the donated chromosome must lie, not only within a sex factor, but within the actual sex factor responsible for conjugation.

Several workers have tested the ability of various plasmids to restore donor ability to defective Hfr strains. Superinfection with normal F-*lac* gave occasional diploid strains possessing the same donor property as the original Hfr (44). Hirota, Fujii, and Nishimura (99) observed that R100-1, a derepressed mutant of an F-like R factor, almost completely restored the function of defective HfrC, which normally gives more *pro* recombinants than either *his* or *trp* (30). As the bacteria produced F pili, distinguishable by minor structural differences from those determined by R100-1 (166), the R factor might well have been replacing the defect in the F factor. Clowes (34) tested ColI, whose sex factor is not related to F, judging from the I pilus and the *fi* character. Since wild-type ColI is repressed, the frequency of all kinds of recombinants was exceedingly low; nevertheless, there were about 50 times more for *pro* than for *his* or *trp*, just as with normal HfrC. This result, as Clowes pointed out, may signify that ColI is merely providing a conjugation bridge while gene transfer results from an independent event like spontaneous rupture of the chromosome which is most probable where F is inserted. In the Richter $♀_3$ strain, which carries an *sfa* locus consisting of only a fragment of F, simple repair of function may be less likely than when a defective but entire F factor is present. However, F, R100-1 (210), and R1*drd* (151) converted the Richter $♀_3$ strain into a chromosomal donor whose polarity of transfer was the same in each case.

Meynell and Cooke (*in preparation*) tested several different R factors, both F-like and I-like, in defective HfrC, using recipient strains with genetic markers distributed throughout the chromosome. Table 7, which gives the results for *pro*, *his*, and *trp*, shows that the original HfrC

polarity of transfer was present with R1*drd*, an F-like factor, and to a lesser extent with R64*drd*, which belongs to the I-like group, whereas R144*drd*, R163*drd* (both I-like), and R192*drd* (F-like) produced equal numbers of each recombinant class, just as in an F^- strain. The positive result with R1*drd* might be explained by functional repair like that of Hirota et al. (99) with R100-1, but the negative result with R192*drd* shows that not all F-like factors act in this way. With the I-like factors, it was possible to determine which sort of sex pili was formed by the bacteria. R64*drd* or R144*drd* caused defective HfrC to produce only I-like pili, as determined by electron microscopy (A. M. Lawn, *personal communication*) and by sensitivity to donor-specific phages, although normal HfrC carrying these factors produced pili of both kinds. Thus, with R64*drd*, the oriented transfer of the HfrC chromosome occurred even when the pilus was determined by the I-like factor, R64*drd*. If transfer required the genetic material to form a continuous structure with the sex factor, R64*drd* might specifically restore the F-oriented transfer of the chromosome in defective HfC because it had greater homology with F than with the chromosome. Confronted with a chromosome containing an integrated F factor, it might then be preferentially inserted into the continuity of the F factor rather than elsewhere.

Resistance genes. Recombination between resistance genes is observed when two R factors tend to exclude each other and enter an unstable relationship in which one sex factor is ultimately lost. Simultaneous selection for the resistance genes from both factors then yields a stable R^+ minority in which all the resistance genes are found united with the remaining sex factor in a single linkage group, as shown by their cotransduction by phage P1 (156, 223, 227). This is observed only when both R factors are fi^+ or fi^- (227), probably because fi^+ and fi^- factors reproduced unrestrictedly in the same cell so that culture on drug-containing media does not select sufficiently strongly for recombinants. The same reason probably explains the failure to isolate recombinants of R factors and F, except when drug-resistance markers are transduced without their sex factor by phage P22 (223, 228) or phage ϵ_{15} or ϵ_{34} (88), when subsequent transfer of resistance genes by F can be used for selection.

An R factor is transduced as a whole by phage P1 because the length of the transduced fragment is sufficient to include both the resistance genes and the sex factor. The transduced factor is therefore as transmissible as the original. On the other hand, when transduced by phages P22, ϵ_{15}, or ϵ_{34}, the resistance markers are not subsequently transmissible and appear to be integrated in a defective phage genome (57). The difference between these phages and phage P1 presumably arises because shorter fragments are transferred, and, consequently, the resistance genes are transduced without the genome of the sex factor and do not comprise a replicon. Therefore, the only transduced resistance markers which are replicated in the recipient are those associated with a new replicon, namely, the genome of the transducing phage which becomes defective.

Transductants obtained with phages ϵ_{15} and ϵ_{34} form stable associations with the F′ factor, F 13, in which the resistance genes are evidently united with F, because they are jointly transduced by phage P1. These recombinants also resemble F, and differ from the original R factor, by being eliminated during growth in the presence of acridines (89). Recombination is not confined to exchange of resistance determinants, for it may also occur between genes of the sex factor proper. For example, transduction of resistance from one R factor by phage P22 gave an exceptional R^+ clone which conjugated at very low frequency; when the transduced factor was transferred to F^+ bacteria, it recombined with F to yield a hybrid factor. This resembled both F, in being unrepressed and in excluding other F factors, and R, in not being eliminated by acridines (228).

Neither is recombination of resistance genes limited to changing from one sex factor to another for they can be stably linked to a fully functional genome of phage ϵ_{15} (119) or phage P1 (124). Drug resistance is then expressed by every phage-infected bacterium, and appears as an instance of phage conversion. The resistance genes in the phage-infected bacterium can then be subsequently restored to linkage with a sex factor (123).

Recombination is of potential value in mapping resistance genes within R factors. Point mutations within the chloramphenicol-resistance or tetracycline-resistance genes have been mapped with two factors, one of which lacked sex factor activity and did not exclude the other (93, 95, 155). Interrupted mating, applied so successfully to Hfr donors (116) and to the chromosomal sequences of long F′ factors (26, 101), cannot, however, be used to map R factors transferred to R^- recipients, because the method depends on there being a homologous region in the recipient's genome to rescue incoming genes by recombination. This presumably accounts for the observation that the first resistant bacteria to appear in the recipient population are found to possess the entire R factor, not individual resistance genes (222).

Interactions between transmissible and nontransmissible plasmids. A special instance of genetic

interaction concerns the transfer of a "nontransmissible" plasmid by a second plasmid possessing sex factor activity. Any stable plasmid comprises a replicon, whose replication starts at a specialized gene or "replicator" (112). With transmissible plasmids, the replicator is likely to be a part of the sex factor, any accompanying genes, like the resistance genes of an R factor, being reproduced with it in the same manner as the chromosomal sequence of an F′ factor. On the other hand, the nature of nontransmissible plasmids is unknown. Some may comprise defective sex factors which have lost their ability to promote conjugation. Others may contain a defective phage genome, not easily recognized as such following loss to its phagelike functions; resistance genes derived from R factors have been shown to form stable associations with intact phage P1 (122, 123), defective phage ϵ_{15} (119), or P22 (57). Nontransmissible plasmids include many ColE factors, other plasmids determining a similar colicin in *Salmonella* strains, and numerous factors for other colicins (74, 164, 177). All these are often transferred at considerable frequency when conjugation is brought about by another plasmid with sex factor activity. Co-transfer could occur by complementation or recombination, and, if either is a criterion of relationship, a given sex factor should transfer some nontransmissible plasmids more readily than others. There are few experiments that allow this question to be decided. Nagel de Zwaig (164) found that ColE1-K30 was readily transferred by F, and Clowes (35) collected a number of reports suggesting that this plasmid is efficiently transferred at conjugation brought about by either F or ColI, although Watanabe (224) observed that transfer of ColE1-K30 from a donor carrying both F and ColI was prevented if F transfer (but not ColI transfer) was suppressed by an *fi*$^+$ R factor. ColE2-P9, on the other hand, was transferred at much higher frequency with ColI than with F (35, 58, 98). ColE3-CA38 is poorly transferred with either F or ColI (98).

Origin of Resistance Genes

The source of individual resistance genes might be identifiable by genetic or by biochemical means. The available evidence is scanty but strongly suggests that many or all of these genes did not arise as mutations in the chromosome of *Salmonella*, *Shigella*, or *E. coli*, in which they are now commonly found. Genetically, they do not exhibit homology with the chromosome, for chromosomal integration of resistance genes has never been unequivocally demonstrated except through the intermediary of phage P22 (58) or ϵ_{15} (90) after transduction by these phages. Homology does not appear to have been assessed directly by DNA hybridization.

The comparative biochemistry of drug resistance in R$^+$ and R$^-$ bacteria should help to reveal the origins of R factor resistance genes. R factors determine penicillinases in *E. coli* which differ from the usual penicillinase of this species; a variety of different penicillinases are determined by different R factors, and one resembles a *Klebsiella* penicillinase (50, 52, 64). With kanamycin and chloramphenicol, enzymes inactivating the drugs by acetylation have been demonstrated (69, 194); although R$^-$ strains of enteric bacteria have been shown to acetylate small amounts of chloramphenicol (194), the effect with R factors appears to be brought about by an R-determined enzyme (W. V. Shaw, *personal communication*). Mutation in *E. coli* to high-level resistance to streptomycin in one step is associated with the formation of resistant ribosomes (24), and the chromosomal *str-r* gene is recessive to *str-s* (131): the *str-r* gene of an R factor is evidently dominant to *str-s* in the chromosome and the ribosomes remain streptomycin-sensitive (185). However, many other chromosomal mutations lead to lower levels of resistance, comparable to those of R factors, by mechanisms which are still unknown; moreover, if a recessive *str-r* gene were carried by an R factor, it would probably go undetected. Exclusion of drug from the cell has been postulated to account for resistance to tetracycline, in the same way as occurs with bacterial mutants isolated in the laboratory (72, 73, 107, 108, 216).

DISCUSSION

Of all the criteria of relationship discussed in the second half of this review, superinfection immunity is by far the most significant. The results obtained with pairs of marked derivatives of a given plasmid, like two segregants of an R factor (224) or two F′ derivatives of F (189), show that two related plasmids only rarely coexist stably in the same cell. More importantly, although an *fi*$^+$ and an *fi*$^-$ R factor coexist readily, any two factors of the same *fi* type are rarely able to do so. It follows that the *fi* character of an R factor is found in nature to be associated with a specific requirement for replication which cannot be satisfied if the host bacterium already carries another R factor of the same *fi* type. We have seen that the *fi* character reflects the specificity of the mechanisms regulating synthesis of the sex pilus, and also that it is associated with specific structural genes for the pilus, since the *fi*$^+$ character is found with F-like sex pili and the *fi*$^-$character with I-like pili (Table 4). Thus, naturally occurring R factors exhibit an association of three functions: replication, regulation of pilus

formation, and pilus structure. We have avoided a precise definition of "sex factor" up to this point, but it will now be evident that it comprises not only the structural and regulatory genes of the sex pilus but also the determinants of replication, i.e., the initiator and replicator of the replicon model (114), as is clear from the role of F in determining the polarity of the Hfr chromosome (2). Since sex piliso far appear to be limited to a few, perhaps only two, types, there may prove to be correspondingly few specificities of replication, as revealed by superinfection immunity.

The question then arises as to whether this association between a specific replication mechanism and specific pilus genes in each *fi* class is obligatory or merely fortuitous. Genetic replication and pilus formation are wholly dissimilar processes and, although the physiology of transfer may require their genetic determinants to be linked (2, 81, 112), one would expect these determinants to behave independently in crosses. Their association in naturally occurring plasmids may therefore have no more significance than the associations of unrelated characters used in taxonomy. Exceptional plasmids are therefore to be expected, as in the association of an F-like replication system with an I-like pilus, or by the appearance of variant F-like or I-like factors. The structural genes for the pilus of the F-like factor, F_0-*lac*, are atypical in adsorbing filamentous but not isometric F phages. F itself provides an outstanding instance of a factor with an atypical specificity of replication for, although it is the prototype of the F-like class of sex factors, it is exceptional in failing to exhibit superinfection immunity with other members of this class, such as fi^+ R factors. Moreover, F and ColV have two other properties that mark them off: they are naturally derepressed and they are relatively readily eliminated by growth in the presence of acridines (117, 219). The fact that it is F in particular that is aberrant is striking, but may be no more than a historical accident, in that F chanced to be the first sex factor to be identified.

One's view of the nature and origins of R factors is largely determined by one's view of plasmids in general. Isolated genes are incapable of replication (138, 208), and the ability of resistance genes to form a stable association with their bacterial host therefore depends on their linkage to a replicon. This may sometimes be recognizable either as a bacterial sex factor or as a phage genome, examples being P1 or ϵ_{15} converting particles (119, 122), P22 transducing particles (57), and, possibly, staphylococcal plasmids (183). Or it may have no identifiable properties other than the ability to ensure replication of its associated genes, as appears to be the case with ColE2 (177) and some resistance genes (10). The formation of any stable complex plasmid is therefore conditional on the prior existence of a replicon. This may be the sex factor in the case of some transmissible plasmids, although others may result from the integration of a sex factor with a second replicon comprising an otherwise nontransmissible plasmid, in the same way that the Hfr chromosome is formed by integration of two independent replicons, namely, F and the bacterial genome. It is because sex factors appear to be of only a few kinds, despite the variety of characters that are transmissible, that they are of greater value in assessing relationships than the characters themselves. The replicons forming the basis of nontransmissible plasmids may prove to be equally valuable but, at present, their nature is unknown.

Colicin determinants may differ radically in their nature from other genes found on plasmids. Although colicins are defined by their property of killing *E. coli*, they are probably a heterogeneous collection of agents of varied origin. The most revealing division may be between those which are nontransmissible and those, like V, B, and I, associated with sex factor activity. The former could well be components of defective phage (20, 187, 212) and the Col factor a defective phage genome, unable to give infective progeny but still able to bring about synthesis of lethal components of the particle (76). Thus, it may be significant that the nontransmissible factors E1, E2, and E3, but not the transmissible factors I and V, share with phage the property that their synthesis is lethal to the bacterium (98, 176, 231). The transmissible factors, I, B, and V, stand in contrast, for their lethal synthesis has not been demonstrated with certainty. Colicins in the transmissible group may be related to functions involved in bacterial conjugation (182), and their determinants should therefore not be regarded as merely linked to the sex factor in the way that resistance genes are present in an R factor, but as an integral part of the sex factor proper. Support for this view comes from the behavior of derepressed mutants of two R factors which also determine formation of colicin Ib. In each case, the amount of colicin produced was directly related to the extent of derepression of pilus formation, suggesting that both were subject to the same system of regulation (E. Meynell, *unpublished data*). No more copies of the R factor were present than in the wild-type strain, judging from the efficiency with which resistance genes were transduced by phage P1 (17). This effect was specific for colicin synthesis, as derepressed mutants of another R factor, determining penicillin resistance, which were selected for increased conjugating

ability, did not form more penicillinase than the wild-type parent. Its specificity is also shown by its mutational origin, as distinct from the increase in colicinogeny and conjugating ability which follow a nonspecific treatment like UV irradiation (5, 36). The coordinate derepression of these two functions in mutant R factors may therefore indicate a relationship between colicin I and conjugation.

Bacterial plasmids are associated with such a variety of functions that, at first sight, they might appear to defy classification in any but the most superficial descriptive terms. This variety is misleading, however, and disappears once plasmids are considered in genetic, not physiological, terms and as subject to the same rules as any other genetic element. A stable plasmid is necessarily a replicon. If it is transmissible, it necessarily contains a sex factor. Immediately the fundamental properties of transmissible plasmids are considered, consistent and logical relationships appear. Relationships between F, phage, converting particles, F′, col factors, and R factors have long been noted in the literature (76, 96, 97, 116, 138, 221). We have seen here that functionally diverse transmissible plasmids can have similar sex factors; to emphasize functional differences between complex genetic elements may be, therefore, in the context of their evolutionary relationships, to concentrate on their trivial properties, the determinants which chance to be linked to the replicon and which are continually changing by recombination and segregation. The critical step in the evolution of plasmids, both transmissible and nontransmissible, must have been the appearance of the first replicon capable of an independent nonchromosomal existence. Once this was formed, experiment shows how rapidly complex plasmids can be assembled, even within the limited time span of the laboratory. The nature of the primordial replicon is as obscure as that of bacteria themselves. It might have been a duplicate of the chromosomal replicator and initiator genes, so that its host was a partial diploid. It might equally well have evolved from an extrachromosomal genetic element like a centrosome, which was once concerned in bacterial mitosis. If, in bacteria, chromosomal replication once took place upon a mitotic spindle, as in higher cells, then the substitution of the cell membrane in ensuring the partition of daughter chromosomes (47, 112) would render superfluous the genetic elements of the spindle. Perhaps these are the ancestors of the plasmids we meet today.

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LITERATURE CITED

1. ADELBERG, E. A., AND S. N. BURNS. 1960. Genetic variation in the sex factor of *Escherichia coli*. J. Bacteriol. **79**:321–330.
2. ADELBERG, E. A., AND J. PITTARD. 1965. Chromosome transfer in bacterial conjugation. Bacteriol. Rev. **29**:161–172.
3. AKIBA, T. 1959. Mechanism of development of resistance in shigella. Proc. 15th Gen. Meet. Japan. Med. Assoc. **5**:299–305.
4. AKIBA, T., K. KOYAMA, Y. ISHIKI, S. KIMURA, AND T. FUKUSHIMA. 1960. On the mechanism of the development of multiple-drug-resistant clones of shigella. Japan. J. Microbiol. **4**:219–227.
5. AMATI, P. 1964. Vegetative multiplication of colicinogenic factors after induction in *Escherichia coli*. J. Mol. Biol. **8**:239–246.
6. ANDERSON, E. S. 1965. Origin of transferable drug-resistance factors in the enterobacteriaceae. Brit. Med. J. **2**:1289–1291.
7. ANDERSON, E. S. 1967. Facteurs de transfert et resistance aux antibiotiques chez les entérobactéries. Ann. Inst. Pasteur **112**:547–563.
8. ANDERSON, E. S., AND N. DATTA. 1965. Resistance to penicillins and its transfer in enterobacteriaceae. Lancet **1**:407–409.
9. ANDERSON, E. S., AND A. FRASER. 1956. The statistical distribution of phenotypically modifiable particles and host-range mutants in populations of Vi-phage II. J. Gen. Microbiol. **15**:225–239.
10. ANDERSON, E. S., AND M. J. LEWIS. 1965. Drug resistance and its transfer in *Salmonella typhimurium*. Nature **206**:579–583.
11. ANDERSON, E. S., AND D. PERRET. 1967. Mobilization of transduced tetracycline resistance by the transfer factor in *Salmonella typhimurium* and *S. typhi*. Nature **214**:810–811.
12. ARBER, W. 1965. Host-controlled modification of bacteriophage. Ann. Rev. Microbiol. **19**:365–378.
13. ARBER, W. 1966. Host specificity of DNA produced by *Escherichia coli*. 9. Host-controlled modification of bacteriophage fd. J. Mol. Biol. **20**:483–496.
14. ARBER, W., AND M. L. MORSE. 1965. Host specificity of DNA produced by *Escherichia coli*. Effects on bacterial conjugation. Genetics **51**:137–148.
15. BARON, L. S., W. F. CAREY, AND W. M. SPILMAN. 1959. Characteristics of high frequency of recombination (HFR) strain of *Salmonella typhosa* compatible with *Salmonella*, *Shigella*

and *Escherichia* species. Proc. Natl. Acad. Sci. U.S. **45**:1752–1757.
16. BARON, L. S., AND S. FALKOW. 1961. Genetic transfer of episomes from *Salmonella typhosa* to *Vibrio cholerae*. Genetics **46**:849.
17. BERG, C. M., AND L. G. CARO. 1967. Chromosome replication in *Escherichia coli*. 1. Lack of influence of the integrated F factor. J. Mol. Biol. **29**:419–431.
18. BERNSTEIN, H. L. 1958. Fertility factors in *Escherichia coli*. Symp. Soc. Exptl. Biol. **12**:93–103.
19. BERTANI, G. 1954. Studies on lysogenesis. III. Superinfection of lysogenic Shigella dysenteriae with temperate mutants of the carried phage. J. Bacteriol. **67**:696–707.
20. BRADLEY, D. E., AND C. A. DEWAR. 1966. The structure of phage-like objects associated with non-induced bacteriocinogenic bacteria. J. Gen. Microbiol. **45**:399–408.
21. BRINTON, C. C. 1959. Non-flagellar appendages of bacteria. Nature **183**:782–786.
22. BRINTON, C. C. 1965. The structure, function, synthesis and genetic control of bacterial pili and a molecular model for DNA and RNA transport in gram-negative bacteria. Trans. N.Y. Acad. Sci. **27**:1003–1054.
23. BRINTON, C. C., P. GEMSKI, AND J. CARNAHAN. 1964. A new type of bacterial pilus genetically controlled by the fertility factor of *E. coli* K12 and its role in chromosome transfer. Proc. Natl. Acad. Sci. U.S. **52**:776–783.
24. BROCK, T. D. 1966. Streptomycin. Symp. Soc. Gen. Microbiol. **16**:131–168.
25. BRODA, P. 1967. The formation of Hfr strains in *Escherichia coli* K12. Genet. Res. **9**:35–47.
26. BRODA, P., J. R. BECKWITH, AND J. SCAIFE. 1964. The characterisation of a new type of F-prime factor in *Escherichia coli* K12. Genet. Res. **5**:489–494.
27. CAIRNS, J. 1963. The chromosome of *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. **28**:43–46.
28. CAMPBELL, A. M. 1962. Episomes. Advan. Genet. **11**:101–145.
29. CARO, L. G., AND M. SCHNÖS. 1966. The attachment of the male-specific bacteriophage F1 to sensitive strains of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. **56**:126–132.
30. CAVALLI-SFORZA, L. L. 1950. La sessualita nei batteri. Boll. Inst. Sieroterap. Milan **29**:281–289.
31. CAVALLI, L. L., J. LEDERBERG, AND E. M. LEDERBERG. 1953. An infective factor controlling sex compatibility in *Bacterium coli*. J. Gen. Microbiol. **8**:89–103.
32. CHANGEUX, J. P. 1960. Sur l'expression biochimique de déterminants génétiques d'*Escherichia coli* introduits chez *Salmonella typhimurium*. Compt. Rend. **250**:1575–1577.
33. CLARK, A. J., AND E. A. ADELBERG. 1962. Bacterial conjugation. Ann. Rev. Microbiol. **16**:289–319.
34. CLOWES, R. C. 1963. Co-operation of two sex factors in *E. coli* K12. Biochem. Biophys. Res. Commun. **13**:449–454.
35. CLOWES, R. C. 1964. Transfert génétique des facteurs colicinogènes. Ann. Inst. Pasteur **107** (no. 5, suppl.):74–92.
36. CLOWES, R. C. 1965. Colicin factors as sex factors. Symp. Biol. Hung. **6**:85–95.
37. CLOWES, R. C., AND E. E. M. MOODY. 1966. Chromosomal transfer from "recombination-deficient" strains of *Escherichia coli* K12. Genetics **53**:717–726.
38. COLSON, C., S. W. GLOVER, N. SYMONDS, AND K. A. STACEY. 1965. The location of genes for host-controlled modification and restriction in *Escherichia coli* K12. Genetics **52**:1043–1050.
39. COOPER, S., AND N. D. ZINDER. 1962. The growth of an RNA bacteriophage: the role of DNA synthesis. Virology **18**:405–411.
40. CRAWFORD, E. M., AND R. F. GESTELAND. 1964. The adsorption of bacteriophage R-17. Virology **22**:165–167.
41. CUZIN, F. 1962. Multiplication autonome de l'épisome sexual d'*Escherichia coli* K12 dans une souche Hfr. Compt. Rend. **254**:4211–4213.
42. CUZIN, F. 1962. Mutants défectifs de l'épisome sexuel chez *Escherichia coli* K12. Compt. Rend. **255**:1149–1151.
43. CUZIN, F., AND F. JACOB. 1963. Intégration reversible de l'épisome sexuel F′ chez *Escherichia coli* K12. Compt. Rend. **257**:795–797.
44. CUZIN, F., AND F. JACOB. 1965. Analyse génétique fonctionelle de l'épisome sexuel d'*Escherichia coli* K12. Compt. Rend. **260**:2087–2090.
45. CUZIN, F., AND F. JACOB. 1967. Mutations de l'épisome F d'*Escherichia coli* K12. 1. Mutations défectives. Ann. Inst. Pasteur **112**:1–9.
46. CUZIN, F., AND F. JACOB. 1967. Mutations de l'épisome F d'*Escherichia coli* K12. 2. Mutants à réplication thermosensible. Ann. Inst. Pasteur **112**:397–418.
47. CUZIN, F., AND F. JACOB. 1967. Existence chez *Escherichia coli* K12 d'une unité génétique de transmission formée de différents réplicons. Ann. Inst. Pasteur **112**:529–545.
48. DATTA, N. 1962. Transmissible drug resistance in an epidemic strain of *Salmonella typhimurium*. J. Hyg. **60**:301–310.
49. DATTA, N. 1965. Infectious drug resistance. Brit. Med. Bull. **21**:254–259.
50. DATTA, N., AND P. KONTOMICHALOU. 1965. Penicillinase synthesis controlled by infectious R factors in *Enterobacteriaceae*. Nature **208**:239–241.
51. DATTA, N., A. M. LAWN, AND E. MEYNELL. 1966. The relationship of F type piliation and F phage sensitivity to drug resistance transfer in R^+F^- *Escherichia coli* K12. J. Gen. Microbiol. **45**:365–376.
52. DATTA, N., AND M. H. RICHMOND. 1966. The purification and properties of a penicillinase whose synthesis is mediated by an R factor in *Escherichia coli*. Biochem. J. **98**:204–209.
53. DETTORI, R., G. A. MACCACARO, AND G. L.

PICCININ. 1961. Sex specific bacteriophages of *Escherichia coli* K12. Giorn. Microbiol. **9**:141–150.

54. DETTORI, R., G. A. MACCACARO, AND M. TURRI. 1963. Sex specific bacteriophages of *E. coli* K12. IV. Host specificity, pattern of lysis and lethality of phage $\mu 2$. Giorn. Microbiol. **11**:15–34.
55. DETTORI, R., AND M. G. NERI. 1965. Batteriofago filamentoso specifico per cellule Hfr ed F^+ di *E. coli* K12. Giorn. Microbiol. **13**:111–121.
56. DRISKELL-ZAMENHOFF, P. J., AND E. A. ADELBERG. 1963. Studies on the chemical nature and size of sex factors of *Escherichia coli*. J. Mol. Biol. **6**:483–497.
57. DUBNAU, E., AND B. A. D. STOCKER. 1964. Genetics of plasmids in *Salmonella typhimurium*. Nature **204**:1112–1113.
58. DUBNAU, E., AND B. A. D. STOCKER. 1967. Behaviour of three colicine factors and an R (drug-resistance) factor in Hfr crosses in *Salmonella typhimurium*. Genet. Res. **9**:283–297.
59. DUGUID, J. P., E. S. ANDERSON, AND E. CAMPBELL. 1966. Fimbriae and adhesive properties in salmonellae. J. Pathol. Bacteriol. **92**:107–138.
60. DUGUID, J. P., I. W. SMITH, G. DEMPSTER, AND P. N. EDMUNDS. 1955. Nonflagellar filamentous appendages ("fimbriae") and haemagglutinating activity in *Bacterium coli*. J. Pathol. Bacteriol. **70**:335–348.
61. ECHOLS, H. 1963. Properties of F′ strains of *Escherichia coli* superinfected with F-lactose and F-galactose episomes. J. Bacteriol. **85**: 262–268.
62. EGAWA, R., AND Y. HIROTA. 1962. Inhibition of fertility by multiple drug-resistance factor in *Escherichia coli* K12. Japan. J. Genet. **37**:66–69.
63. EGAWA, R., T. SAWAI, AND S. MITSUHASHI. 1967. Drug resistance of enteric bacteria. XII. Unique substrate specificity of penicillinase produced by R factor. Japan. J. Microbiol. **11**:173–178.
64. ENGELHARDT, D. L., AND N. D. ZINDER. 1964. Host-dependent mutants of the bacteriophage f2. 3. Infective RNA. Virology **23**:582–587.
65. FALKOW, S., AND L. S. BARON. 1962. Episomic element in a strain of *Salmonella typhosa*. J. Bacteriol. **84**:581–589.
66. FALKOW, S., AND R. V. CITARELLA. 1965. Molecular homology of F-merogenote DNA. J. Mol. Biol. **12**:138–151.
67. FALKOW, S., R. V. CITARELLA, J. A. WOHLHEITER, AND T. WATANABE. 1966. The molecular nature of R factors. J. Mol. Biol. **17**:102–116.
68. FALKOW, S., J. A. WOHLHEITER, R. V. CITARELLA, AND L. S. BARON. 1964. Transfer of episomic elements to *Proteus*. I. Transfer of F-linked chromosomal determinants. J. Bacteriol. **87**:209–219.
69. FALKOW, S., J. A. WOHLHEITER, R. V. CITARELLA, AND L. S. BARON. 1964. Transfer of episomic elements to *Proteus*. II. Nature of lac^+ *Proteus* strains isolated from clinical specimens. J. Bacteriol. **88**:1598–1601.
70. FERRANT, W. N., AND A. J. H. TOMLINSON. 1966. Some studies on the epidemiology of sonne dysentery. Changes in colicine type and antibiotic resistance between 1956 and 1965. J. Hyg. **64**:287–303.
71. FELIX, A. 1955. World survey of typhoid and paratyphoid-B phage types. Bull. World Health Org. **13**:109–170.
72. FRANKLIN, T. J. 1967. Resistance of *Escherichia coli* to tetracyclines. Changes in permeability to tetracyclines in *Escherichia coli* bearing transferable resistance factors. Biochem. J. **105**:371–378.
73. FRANKLIN, T. J., AND A. GODFREY. 1965. Resistance of *E. coli* to tetracyclines. Biochem. J. **94**:54–60.
74. FREDERICQ, P. 1956. Recherches sur la fréquence des souches transductrices des proprietés colicinogènes. Compt. Rend. Soc. Biol. **150**:1036–1039.
75. FREDERICQ, P. 1957. Colicins. Ann. Rev. Microbiol. **11**:7–22.
76. FREDERICQ, P. 1963. On the nature of colicinogenic factors: a review. J. Theoret. Biol. **4**:159–165.
77. FREDERICQ, P. 1964. Colicines et colicinogénie. Ann. Inst. Pasteur **107** (suppl. #5):7–17.
78. FURNESS, G., AND D. ROWLEY. 1957. The presence of the transmissible agent F in nonrecombining strains of *Escherichia coli*. J. Gen. Microbiol. **17**:550–561.
79. GALLUCCI, E., AND G. SIRONI. 1964. A defective sex factor in *E. coli* K12. Giorn. Microbiol. **12**:175–182.
80. GINOZA, H. S., AND T. S. MATNEY. 1963. Transmission of a resistance transfer factor from *Escherichia coli* to two species of *Pasteurella*. J. Bacteriol. **85**:1177–1178.
81. GROSS, J. D., AND L. G. CARO. 1966. DNA transfer in bacterial conjugation. J. Mol. Biol. **16**:269–284.
82. GUINÉE, P. A. M. 1965. Present day problems of intensive animal husbandry. Cyanamide of Great Britain Ltd., London.
83. GUINÉE, P. A. M., R. T. SCHOLTENS, AND H. M. C. C. WILLEMS. 1967. Influence of resistance factors on the phage types of *Salmonella panama*. Antonie van Leeuwenhoek J. Microbiol. Serol. **33**:30–40.
84. DE HAAN, P. G., AND A. H. STOUTHAMER, 1963. F-prime transfer and multiplication of sexduced cells. Genet. Res. **4**:30–41.
85. HAKURA, A., N. OTSUJI, AND Y. HIROTA. 1964. A temperate phage specific for female strains of *Escherichia coli* K12. J. Gen. Microbiol. **35**:69–73.
86. HAMON, Y. 1964. Les bactériocines. Ann. Inst. Pasteur **107** (no. 5, suppl.):18–53.
87. HAMON, Y., AND Y. PERON. 1966. La propriété bacteriocinogène dans la tribu des *Sal-*

monelleae. 1. Les bactériocines des *Salmonella*. Ann. Inst. Pasteur **110**:389–402.

88. HARADA, K., M. KAMEDA, M. SUZUKI, AND S. MITSUHASHI. 1963. Drug resistance of enteric bacteria. II. Transduction of transmissible drug-resistance (R) factors with phage epsilon. J. Bacteriol. **86**:1332–1338.
89. HARADA, K., M. KAMEDA, M. SUZUKI, AND S. MITSUHASHI. 1964. Drug resistance of enteric bacteria. III. Acquisition of transferability of nontransmissible R(TC) factor in cooperation with F factor and formation of FR(TC). J. Bacteriol. **88**:1257–1265.
90. HARADA, K., M. KAMEDA, M. SUZUKI, S. SHIGEHARA, AND S. MITSUHASHI. 1967. Drug resistance of enteric bacteria. VIII. Chromosomal location of nontransferable R factor in *Escherichia coli*. J. Bacteriol. **93**:1236–1241.
91. HARADA, K., M. SUZUKI, M. KAMEDA, AND S. MITSUHASHI. 1960. On the drug-resistance of enteric bacteria. 2. Transmission of the drug-resistance among *Enterobacteriaceae*. Japan. J. Exptl. Med. **30**:289–299.
92. HARDY, P. H., AND E. E. NELL. 1967. Four transfer factors in a single bacterial strain. Nature **214**:414–415.
93. HASHIMOTO, H., AND Y. HIROTA. 1966. Gene recombination and segregation of resistance factor R in *Escherichia coli*. J. Bacteriol. **91**:51–62.
94. HASHIMOTO, H., AND S. MITSUHASHI. 1966. Drug resistance of enteric bacteria. VII. Recombination of R factors with tetracycline-sensitive mutants. J. Bacteriol. **92**:1351–1356.
95. HAYES, W. 1952. Recombination in *Bact. coli* K12: unidirectional transfer of genetic material. Nature **169**:118.
96. HAYES, W. 1953. Observations on a transmissible agent determining sexual differentiation in *Bact. coli*. J. Gen. Microbiol. **8**:72–88.
97. HAYES, W. 1964. The genetics of bacteria and their viruses. Blackwell Scientific Publications, Oxford, England.
98. HERSCHMAN, H. R., AND D. R. HELINSKI. 1967. Comparative study of the events associated with colicin induction. J. Bacteriol. **94**:691–699.
99. HIROTA, Y., T. FUJII, AND Y. NISHIMURA. 1966. Loss and repair of conjugal fertility and infectivity of the resistance factor and sex factor in *Escherichia coli*. J. Bacteriol. **91**:1298–1304.
100. HIROTA, Y., Y. NISHIMURA. F. ØRSKOV, AND I. ØRSKOV. 1964. Effect of drug-resistance factor R on the F properties of *Escherichia coli*. J. Bacteriol. **87**:341–351.
101. HIROTA, Y., AND P. H. A. SNEATH. 1961. F′ and F mediated transduction in *Escherichia coli* K12. Japan. J. Genet. **36**:307–318.
102. HOFFMANN-BERLING, H., H. C. KAERNER, AND R. KNIPPERS. 1966. Small bacteriophages. Advan. Virus Res. **12**:329–365.
103. HOFFMANN-BERLING, H., AND R. MAZÉ. 1964. Release of male-specific bacteriophages from surviving host bacteria. Virology **22**:305–313.
104. HOFSCHNEIDER, P. H. 1963. Untersuchungen über "Kleine" *E. coli* K12 Bakteriophagen. 1. Mitt: Die Isolierung und einige Eigenschaften der Kleinen. Z. Naturforsch **18b**:203–205.
105. IPPEN, K. A., AND R. C. VALENTINE. 1967. The sex hair of *E. coli* as sensory fiber, conjugation tube or mating arm? Biochem. Biophys. Res. Commun. **27**:674–675.
106. ISHIBASHI, M. 1967. F pilus as f^+ antigen. J. Bacteriol. **93**:379–389.
107. IZAKI, K., AND K. ARIMA. 1963. Disappearance of oxytetracycline accumulation in the cells of multiple drug-resistant *Escherichia coli*. Nature **200**:384–385.
108. IZAKI, K., K. KIUCHI, AND K. ARIMA. 1966. Specificity and mechanism of tetracycline resistance in a multiple drug resistant strain of *Escherichia coli*. J. Bacteriol. **91**:628–633.
109. JACOB, F. 1954. Les bactéries lysogènes et la notion de provirus. Masson et Cie., Paris.
110. JACOB, F., AND E. A. ADELBERG. 1959. Transfert de charactères génétiques par incorporation au facteur sexuel d'*Escherichia coli*. Compt. Rend. **249**:189–191.
111. JACOB, F., AND S. BRENNER. 1963. Sur la régulation de la synthèse du DNA chez les bactéries: l'hypothèse du replicon. Compt. Rend. **256**: 298–300.
112. JACOB, F., S. BRENNER, AND F. CUZIN. 1963. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **28**: 329–348.
113. JACOB, F., AND J. MONOD. 1961. Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. **3**:318–356.
114. JACOB, F., AND E. L. WOLLMAN. 1956. Recombinaison génétique et mutants de fertilité chez *Escherichia coli*. Compt. Rend. **242**: 303–306.
115. JACOB, F., AND E. L. WOLLMAN. 1957. Analyse des groupes de liaison génétique de différentes souches donatrices d'*Escherichia coli* K12. Compt. Rend. **245**: 1840–1843.
116. JACOB, F., AND E. L. WOLLMAN. 1961. Sexuality and the genetics of bacteria. Academic Press, Inc., New York.
117. KAHN, P., AND D. R. HELINSKI. 1964. Relationship between colicinogenic factors E_1 and V and an F factor in *Escherichia coli*. J. Bacteriol. **88**:1573–1579.
118. KAHN, P. L., AND D. R. HELINSKI. 1965. Interaction between colicinogenic factor V and the integrated F factor in an Hfr strain of *Escherichia coli* J. Bacteriol. **90**:1276–1282.
119. KAMEDA, M., K. HARADA, M. SUZUKI, AND S. MITSUHASHI. 1965. Drug resistance of enteric bacteria. V. High frequency of transduction of R factors with bacteriophage epsilon. J. Bacteriol. **90**:1174–1181.
120. KATO, Y., M. HANAOKA, AND T. AMANO. 1962. The elimination of a colicinogenic factor by a

drug-resistance transferring factor in *Escherichia coli* K_{235}. Biken's J. **5**:77–86.

121. KITAMOTO, O., N. KASAI, K. FUKAYA, AND A. KAWASHMA. 1956. Drug-sensitivity of the *Shigella* strains isolated in 1955. J. Japan. Assoc. Infectious Diseases **30**:403–404.
122. KONDO, E., AND S. MITSUHASHI. 1964. Drug resistance of enteric bacteria. IV. Active transducing bacteriophage P1 *CM* produced by the combination of R factor with bacteriophage P1. J. Bacteriol. **88**:1266–1276.
123. KONDO, E., AND S. MITSUHASHI. 1966. Drug resistance of enteric bacteria. VI. Introduction of bacteriophage P1 *CM* into *Salmonella typhi* and formation of P1d*CM* and F-*CM* elements. J. Bacteriol. **91**:1787–1794.
124. KONTOMICHALOU, P. 1967. Studies on resistance transfer factors. Pathol. Microbiol. **30**:71–93.
125. KONTOMICHALOU, P. 1967. Studies on resistance transfer factors. II. Transmissible resistance to eight antibacterial drugs in a strain of *Escherichia coli*. Pathol. Microbiol. **30**:185–200.
126. KUWABARA, S., T. AKIBA, K. KOYAMA, AND T. ARAI. 1963. Transmission of multiple drug-resistance from *Shigella flexneri* to *Vibrio comma* through conjugation. Japan. J. Microbiol. **7**:61–68.
127. LAWN, A. M. 1966. Morphological features of the pili associated with R^+F^- and R^-F^+ bacteria. J. Gen. Microbiol. **45**:377–383.
128. LAWN, A. M. 1967. Simple immunological labelling method for electron microscopy and its application to the study of filamentous appendages of bacteria. Nature **214**:1151–1152.
129. LAWN, A. M., E. MEYNELL, G. G. MEYNELL, AND N. DATTA. 1967. Sex pili and the classification of sex factors in the *Enterobacteriaceae*. Nature **216**:343–346.
130. LEBEK, G. 1963. Über die Enstehung mehrfachresistanter Salmonellen. Ein experimenteller Beitrag. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **188**:494–505.
131. LEDERBERG, J. 1951. Streptomycin resistance: a genetically recessive mutation. J. Bacteriol. **61**:549–550.
132. LEDERBERG, J., L. L. CAVALLI, AND E. M. LEDERBERG. 1952. Sex compatibility in *Escherichia coli*. Genetics **37**:720–730.
133. LEDERBERG, J., AND E. M. LEDERBERG. 1956. Infection and heredity, p. 101–124. *In* D. Rudrick [ed.], Cellular mechanisms in differentiation and growth. Princeton Univ. Press, Princeton, N.J.
134. LEDERBERG, J., AND E. L. TATUM. 1946. Novel genotypes in mixed cultures of biochemical mutants of bacteria. Cold Spring Harbor Symp. Quant. Biol. **11**:113–114.
135. LEDERBERG, S. 1957. Suppression of the multiplication of heterologous bacteriophages in lysogenic bacteria. Virology **3**:496–513.
136. LEWIS, M. J., AND B. A. D. STOCKER. 1965. Properties of some group E colicine factors. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **196**:173–183.
137. LURIA, S. E. 1953. Host-induced modifications of viruses. Cold Spring Harbor Symp. Quant. Biol. **18**:237–244.
138. LURIA, S. E., J. N. ADAMS, AND R. C. TING. 1960. Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. Virology **12**:348–390.
139. MACCACARO, G. A. 1955. Cell surface and fertility in *Escherichia coli*. Nature **176**:125–126.
140. MACCACARO, G. A., AND R. COMOLLI. 1956. Surface properties correlated with sex compatibility in *Escherichia coli*. J. Gen. Microbiol. **15**:121–132.
141. MACFARREN, A. C., AND R. C. CLOWES. 1967. A comparative study of two F-like colicin factors, ColV2 and ColV3, in *Escherichia coli* K-12. J. Bacteriol. **94**:365–377.
142. MÄKELÄ, O., P. H. MÄKELÄ, AND S. SOIKKELLI. 1964. Sex-specificity of the bacteriophage T7. Ann. Med. Exptl. Biol. Fenniae (Helsinki) **42**:188–195.
143. MANTEN, A., P. A. M. GUINÉE, AND E. H. KAMPELMACHER. 1966. Incidence of resistance to tetracycline and chloramphenicol among *Salmonella* bacteria found in the Netherlands in 1963 and 1964. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **200**:13–20.
144. MARÉ, I. J., AND J. N. COETZEE. 1965. The isolation of *Enterobacteriaceae* possessing the property of transmissible multiple-drug resistance. South African Med. J. **39**:864–865.
145. MARMUR, J., R. ROWND, S. FALKOW, L. S. BARON, C. SCHILDKRAUT, AND P. DOTY. 1961. The nature of intergeneric episomal infection. Proc. Natl. Acad. Sci. U.S. **47**:972–979.
146. MATNEY, T. S., E. P. GOLDSCHMIDT, N. S. ERWIN, AND R. A. SCROGGS. 1964. A preliminary map of genomic sites for F-attachment in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **17**:278–281.
147. MATSUSHIRO, A. 1963. Specialized transduction of tryptophan markers in *Escherichia coli* K12 by bacteriophage 80. Virology **19**:475–482.
148. MEYNELL, E., AND N. DATTA. 1965. Functional homology of the sex-factor and resistance transfer factors. Nature **207**:884–885.
149. MEYNELL, E., AND N. DATTA. 1966. The relation of resistance transfer factors to the F factor of *Escherichia coli* K12. Genet. Res. **7**:134–140.
150. MEYNELL, E., AND N. DATTA. 1966. The nature and incidence of conjugation factors in *Escherichia coli*. Genet. Res. **7**:141–148.
151. MEYNELL, E., AND N. DATTA. 1967. Mutant drug resistance factors of high transmissibility. Nature **214**:885–887.
152. MEYNELL, G. G., AND A. M. LAWN. 1967. Sex pili and common pili in the conjugational transfer of colicin factor Ib by *Salmonella typhimurium*. Genet. Res. **9**:359–367.
153. MEYNELL, G. G., AND A. M. LAWN. 1967. The

sex factor of colicin factor Ela. Genet. Res. **10**:323–325.

154. MITSUHASHI, S. 1965. Transmissible drug-resistance factor R. Gunma J. Med. Sci. **14**:169–209.
155. MITSUHASHI, S., K. HARADA, AND H. HASHIMOTO. 1960. Multiple resistance of enteric bacteria and transmission of drug-resistance to other strain by mixed cultivation. Japan. J. Exptl. Med. **30**:179–184.
156. MITSUHASHI, S., K. HARADA, H. HASHIMOTO, M. KAMEDA, AND M. SUZUKI. 1962. Combination of two types of transmissible drug-resistance factors in a host bacterium. J. Bacteriol. **84**:9–16.
157. MITSUHASHI, S., K. HARADA, M. KAMEDA, M. SUZUKI, AND R. EGAWA. 1960. On the drug-resistance of enteric bacteria. 3. Transmission of the drug-resistance from *Shigella* to F^- or Hfr strains of *E. coli* K12. Japan. J. Exptl. Med. **30**:301–306.
158. MITSUHASHI, S., H. HASHIMOTO, K. HARADA, R. EGAWA, AND T. MATSUYAMA. 1961. Studies on the drug-resistnce of enteric bacteria. 7. Type of the transmissible drug-resistances factor. Gunma J. Med. Sci. **10**:59–66.
159. MONK, M., AND R. C. CLOWES. 1964. Transfer of the colicin I factor in *Escherichia coli* K12 and its interaction with the F fertility factor. J. Gen. Microbiol. **36**:365–384.
160. MULCZYK, M., AND J. P. DUGUID. 1966. Influence of the state of fimbriation on transmission of the colicinogenic factor *colI* between strains of *Shigella flexneri*. J. Gen. Microbiol. **45**:459–477.
161. NAGEL DE ZWAIG, R. 1966. Association between colicinogenic and fertility factors. Genetics **54**:381–340.
162. NAGEL DE ZWAIG, R., AND D. N. ANTÓN. 1964. Interactions between colicinogenic factors and fertility factors. Biochem. Biophys. Res. Commun. **17**:358–362.
163. NAGEL DE ZWAIG, R., D. N. ANTÓN, AND J. PUIG. 1962. The genetic control of colicinogenic factors E, I and V. J. Gen. Microbiol. **29**:473–484.
164. NAGEL DE ZWAIG, R., AND J. PUIG. 1964. The genetic behaviour of colicinogenic factor E1. J. Gen. Microbiol. **36**:311–321.
165. NISHIMURA, Y. 1964. Defective mutations of sex compatibility in *Escherichia coli*. Japan. J. Genet. **39**:360.
166. NISHIMURA, Y., M. ISHIBASHI, E. MEYNELL, AND Y. HIROTA. 1967. Specific piliation directed by a fertility factor and a resistance factor of *Escherichia coli*. J. Gen. Microbiol. **49**:89–98.
167. NOVICK, A., AND M. WEINER. 1957. Enzyme induction as an all-or-none phenomenon. Proc. Natl. Acad. Sci. U.S. **43**:553–566.
168. OCHIAI, K., T. YAMANAKA, K. KIMURA, AND O. SAWADA. 1959. Studies on inheritance of drug resistance between *Shigella* strains and *Escherichia coli* strains. Nippon Iji Shimpo **1861**:34–36.
169. OKAMOTO, S., AND Y. SUZUKI. 1965. Chloramphenicol-, dihydrostreptomycin- and kanamycin-inactivating enzymes from multiple drug-resistant *Escherichia coli* carrying episome 'R.' Nature **208**:1301–1303.
170. ØRSKOV, I., AND F. ØRSKOV. 1960. An antigen termed f^+ occurring in F^+ *E. coli*. Acta Pathol. Microbiol. Scand. **48**:37–41.
171. ØRSKOV, F., AND I. ØRSKOV. 1961. The fertility of *Escherichia coli* antigen test strains in crosses with K12. Acta Pathol. Microbiol. Scand. **51**:280–290.
172. ØRSKOV, I., AND F. ØRSKOV. 1966. Episome-carried surface antigen K88 of *Escherichia coli*. I. Transmission of the determinant of the K88 antigen and influence on the transfer of chromosomal markers. J. Bacteriol. **91**:69–75.
173. ØRSKOV, I., F. ØRSKOV, W. J. SOJKA, AND J. M. LEACH. 1961. Simultaneous occurrence of *E. coli* B and L antigens in strains from diseased swine. Acta Pathol. Microbiol. Scand. **53**: 404–422.
174. OZEKI, H. 1965. The behaviour of colicinogenic factors in *Salmonella typhimurium*. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **196**:160–173.
175. OZEKI, H., S. HOWARTH, AND R. CLOWES. 1961. Colicine factors as fertility factors in bacteria. Nature **190**:986–989.
176. OZEKI, H., B. A. D. STOCKER, AND H. DE MARGERIE. 1959. Production of colicine by single bacteria. Nature **184**:337–339.
177. OZEKI, H., B. A. D. STOCKER, AND S. SMITH. 1962. Transmission of colicinogeny between strains of *Salmonella typhimurium* grown together. J. Gen. Microbiol. **28**:671–687.
178. PARDEE, A. B. 1962. The synthesis of enzymes, p. 577–630. *In* I. C. Gunsalus and R. Y. Stanier [ed.], The bacteria, vol. 3. Academic Press, Inc., New York.
179. PARDEE, A. B., F. JACOB, AND J. MONOD. 1959. The genetic control and cytoplasmic expression of 'inducibility' in the synthesis of β-galactosidase by *E. coli*. J. Mol. Biol. **1**:165–178.
180. PUIG, J., AND R. NAGEL DE ZWAIG. 1964. Étude génétique d'un facteur colicinogène B et son influence sur la fertilité des croisements chez *Escherichia coli* K12. Ann. Inst. Pasteur **107**(no. 5, suppl.):115–131.
181. REEVES, P. 1960. Role of Hfr mutants in $F^+ \times F^-$ crosses in *E. coli* K12. Nature **185**:265.
182. REEVES, P. 1965. The bacteriocins. Bacteriol. Rev. **29**:24–45.
183. RICHMOND, M. H. 1965. Penicillinase plasmids in *Staphylococcus aureus*. Brit. Med. Bull. **21**: 260–263.
184. RICHTER, A. 1961. Attachment of wild-type F factor to a specific chromosomal region in a variant strain of *Escherichia coli* K12: the phenomenon of episomic alternation. Genet. Res. **2**:333–345.
185. ROSENKRANZ, H. S. 1964. Basis of streptomycin resistance in *Escherichia coli* with a "multiple

drug resistance" episome. Biochim. Biophys. Acta **80**:342–345.

186. ROWND, R., R. NAKAYA, AND A. NAKAMURA. 1966. Molecular nature of the drug-resistance factors of the enterobacteriaceae. J. Mol. Biol. **17**:376–393.
187. SANDOVAL, H. K., H. C. REILLY, AND B. TANDLER. 1965. Colicin 15: possibly a defective bacteriophage. Nature **205**:522–523.
188. SCAIFE, J. 1966. F-prime factor formation in *E. coli* K12. Genet. Res. **8**:189–196.
189. SCAIFE, J., AND J. D. GROSS. 1962. Inhibition of multiplication of an F-lac factor in Hfr cells of *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **7**:403–407.
190. SCAIFE, J., AND J. D. GROSS. 1963. The mechanism of chromosome mobilization by an F′-prime factor in *Escherichia coli* K12. Genet. Res. **4**:328–331.
191. SCHELL, J., S. W. GLOVER, K. A. STACEY, P. M. A. BRODA, AND N. SYMONDS. 1963. The restriction of phage T3 by certain strains of *Escherichia coli*. Genet. Res. **4**:483–484.
192. SEKIJIMA, Y., AND S. ISEKI. 1966. Serological differences between f^+ antigens in *Escherichia coli* and *Salmonella*. Proc. Japan Acad. **42**: 980–983.
193. SEKIJIMA, Y., AND S. ISEKI. 1966. On the r^+ antigens of bacteria having drug resistance transfer factor R. Proc. Japan Acad. **42**:984–988.
194. SHAW, W. V. 1967. The enzymatic acetylation of chloramphenicol by extracts of R factor-resistant *Escherichia coli*. J. Biol. Chem. **242**:687–693.
195. SICCARDI, A. G. 1966. Colicin resistance associated with resistance factors in *Escherichia coli*. Genet. Res. **8**:219–228.
196. SIGNER, E. R., A. TORRIANI, AND C. LEVINTHAL. 1961. Gene expression in intergeneric merozygotes. Cold. Spring Harbor Symp. Quant. Biol. **26**:31–34.
197. SILVER, S., AND H. OZEKI. 1962. Transfer of deoxyribonucleic acid accompanying the transmission of colicinogenic properties by cell mating. Nature **195**:873–874.
198. SILVERMAN, P. M., H. W. MOBACH, AND R. C. VALENTINE. 1967. Sex hair (F-pili) mutants of *E. coli*. Biochem. Biophys. Res. Commun. **27**:412–416.
199. SKAAR, P. D., A. RICHTER, AND J. LEDERBERG. 1957. Correlated selection for mobility and sex-incompatibility in *Escherichia coli* K12. Proc. Natl. Acad. Sci. U.S. **43**:329–333.
200. SMITH, D. H., AND S. E. ARMOUR. 1966. Transferable R factors in enteric bacteria causing infection of the genito-urinary tract. Lancet **2**:15–18.
201. SMITH, H. W. 1966. The incidence of infective drug resistance in strains of *Escherichia coli* isolated from diseased human beings and domestic animals. J. Hyg. **64**:465–474.
202. SMITH, H. W., AND S. HALLS. 1967. The transmissible nature of the genetic factor in *Escherichia coli* that controls haemolysin production. J. Gen. Microbiol. **47**:153–161.
203. SMITH, S. M., H. OZEKI, AND B. A. D. STOCKER. 1963. Transfer of *colE1* and *colE2* during high-frequency transmission of *colI* in *Salmonella typhimurium*. J. Gen. Microbiol. **33**: 231–242.
204. STIRM, S., F. ØRSKOV, I. ØRSKOV, AND A. BIRCH-ANDERSEN. 1967. Episome-carried surface antigen K88 of *Escherichia coli*. III. Morphology. J. Bacteriol. **93**:740–748.
205. STIRM, S., F. ØRSKOV, I. ØRSKOV, AND B. MANSA. 1967. Episome-carried surface antigen K88 of *Escherichia coli*. II. Isolation and chemical analysis. J. Bacteriol. **93**:731–739.
206. STOCKER, B. A. D. 1966. Heterogeneity of I colicines and I colicine factors. Heredity **21**:166.
207. STOCKER, B. A. D., S. M. SMITH, AND H. OZEKI. 1963. High infectivity of *Salmonella typhimurium* newly infected by the *colI* factor. J. Gen. Microbiol. **30**:201–221.
208. STOCKER, B. A. D., N. D. ZINDER, AND J. LEDERBERG. 1953. Transduction of flagellar characters in *Salmonella*. J. Gen. Microbiol. **9**: 410–433.
209. STROBEL, M., AND M. NOMURA. 1966. Restriction of the growth of bacteriophage BF23 by a colicine (ColI-P9) factor. Virology **28**:763–765.
210. SUGINO, Y., AND Y. HIROTA. 1962. Conjugal fertility associated with resistance factor R in *Escherichia coli*. J. Bacteriol. **84**:902–910.
211. TAKANO, T., T. WATANABE, AND T. FUKASAWA. 1966. Specific inactivation of infectious λ DNA by sonicates of restrictive bacteria with R factors. Biochem. Biophys. Res. Commun. **25**:192–198.
212. TAKEYA, K., Y. MINAMISHIMA, K. AMAKO, AND Y. OHNISHI. 1967. A small rod-shaped pyocin. Virology **31**:166–168.
213. TANAKA, T., H. HASHIMOTO, Y. NAGAI, AND S. MITSUHASHI. 1967. Drug-resistance of enteric bacteria. XI. Isolation of *Shigella* strains in hetero-R state. Japan. J. Microbiol. **11**:155–162.
214. TATUM, E. L., AND J. LEDERBERG. 1947. Gene recombination in the bacterium Escherichia coli. J. Bacteriol. **53**:673–684.
215. TAYLOR, A. L., AND E. A. ADELBERG. 1961. Evidence for a closed linkage group in Hfr males of *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **5**:400–404.
216. UNOWSKY, J., AND M. RACHMELER. 1966. Mechanisms of antibiotic resistance determined by resistance-transfer factors. J. Bacteriol. **92**:358–365; erratum, p. 1857.
217. VALENTINE, R. C., AND M. STRAND. 1965. Complexes of F-pili and RNA bacteriophage. Science **148**:511–513.
218. WALSH, J., AND G. G. MEYNELL. 1967. The isolation of non-excluding mutants of phage P22. J. Gen. Virol. **1**:581–582.
219. WATANABE, T. 1963. Infective heredity of multiple drug resistance in bacteria. Bacteriol. Rev. **27**:87–115.

220. WATANABE, T. 1963. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. VI. High-frequency resistance transfer system in *Escherichia coli*. J. Bacteriol. **85**:788–794.
221. WATANABE, T. 1967. Evolutionary relationships of R factors with other episomes and plasmids. Federation Proc. **26**:23–28.
222. WATANABE, T., AND T. FUKASAWA. 1961. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. I. Transfer of resistance factors by conjugation. J. Bacteriol. **81**:669–678.
223. WATANABE, T., AND T. FUKASAWA. 1961. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. III. Transduction of resistance factors. J. Bacteriol. **82**:202–209.
224. WATANABE, T., AND T. FUKASAWA. 1962. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. IV. Interactions between resistance transfer factor and F-factor in *Escherichia coli* K-12. J. Bacteriol. **83**:727–735.
225. WATANABE, T., T. FUKASAWA, AND T. TAKANO. 1962. Conversion of male bacteria of *Escherichia coli* K12 to resistance to f phages by infection with the episome "Resistance Transfer Factor." Virology **17**:218–219.
226. WATANABE, T., AND K. W. LYANG. 1962. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. V. Spontaneous segregation and recombination of resistance factors in *Salmonella typhimurium*. J. Bacteriol. **84**:422–430.
227. WATANABE, T., H. NISHIDA, C. OGATA, T. ARAI, AND S. SATO. 1964. Episome-mediated transfer of drug resistance in Enterobacteriaceae. VII. Two types of naturally occurring R factors. J. Bacteriol. **88**:716–726.
228. WATANABE, T., AND C. OGATA. 1966. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. IX. Recombination of an R factor with F. J. Bacteriol. **91**:43–50.
229. WATANABE, T., AND M. OKADA. 1964. New type of sex factor-specific bacteriophage of *Escherichia coli*. J. Bacteriol. **87**:727–736.
230. WATANABE, T., T. TAKANO, T. ARAI, H. NISHIDA, AND S. SATO. 1966. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. X. Restriction and modification of phages by *fi*⁻ R factors. J. Bacteriol. **92**:477–486.
231. DEWITT, W., AND D. R. HELINSKI. 1965. Characterization of colicinogenic factor E1 from a non-induced and a mitomycin C-induced *Proteus* strain. J. Mol. Biol. **13**:692–703.
232. WOHLHEITER, J. A., S. FALKOW, R. V. CITARELLA AND L. S. BARON. 1964. Characterization of DNA from a *Proteus* strain harbouring an episome. J. Mol. Biol. **9**:576–588.
233. WOLLMAN, E. L., F. JACOB, AND W. HAYES. 1956. Conjugation and genetic recombination in *Escherichia coli* K12. Cold Spring Harbor Symp. Quant. Biol. **21**:141–162.
234. YOSHIKAWA, M., AND T. AKIBA. 1962. Studies on transferable drug resistance in bacteria. 4. Suppression of plaque formation of phages by the resistance factor. Japan J. Microbiol. **6**:121–132.
235. ZINDER, N. D. 1960. Sexuality and mating in *Salmonella*. Science **131**:924–926.
236. ZINDER, N. D. 1961. A bacteriophage specific for F- *Salmonella* strains. Science **133**:2069–2070.
237. ZINDER, N. D. 1965. RNA phages. Ann. Rev. Microbiol. **19**:455–472.

ERRATA

Extracellular Lipids of Yeasts

FRANK H. STODOLA, MARIA H. DEINEMA, AND J. F. T. SPENCER

Pioneering Laboratory for Microbiological Chemistry, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois 61604; Agricultural University, Wageningen, The Netherlands; and Prairie Regional Laboratory, Saskatoon, Saskatchewan, Canada

Volume 31, no. 3, p. 206: In the legend of Fig. 4, "TE, tubular elements" should be "ME, membranous structures."

Genetic Analysis and Genome Structure in *Streptomyces coelicolor*

DAVID A. HOPWOOD

Institute of Genetics, University of Glasgow, Glasgow, Scotland

Volume 31, no. 4: the tabulated data appearing under the legend to Fig. 5, p. 388, should appear above the legend to Fig. 6, p. 390.